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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:43:19 ON 07 JUL 2004

=> fil .bec,caba,agricola
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.42       0.42

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS, CABA, AGRICOLA'
ENTERED AT 09:44:12 ON 07 JUL 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

13 FILES IN THE FILE LIST

=> s chitinase#
FILE 'MEDLINE'
L1        1739 CHITINASE#

FILE 'SCISEARCH'
L2        3416 CHITINASE#

FILE 'LIFESCI'
L3        1621 CHITINASE#

FILE 'BIOTECHDS'
L4        1016 CHITINASE#

FILE 'BIOSIS'
L5        3923 CHITINASE#

FILE 'EMBASE'
L6        1284 CHITINASE#

FILE 'HCAPLUS'
L7        4720 CHITINASE#

FILE 'NTIS'
L8          30 CHITINASE#

FILE 'ESBIOBASE'
L9        1526 CHITINASE#

FILE 'BIOTECHNO'
L10       1420 CHITINASE#

FILE 'WPIDS'
L11        500 CHITINASE#

FILE 'CABA'
L12       2421 CHITINASE#

FILE 'AGRICOLA'
L13       1368 CHITINASE#

TOTAL FOR ALL FILES
L14      24984 CHITINASE#

=> s barley
FILE 'MEDLINE'
L15       5347 BARLEY

FILE 'SCISEARCH'
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L16      30042 BARLEY

FILE 'LIFESCI'
L17       6393 BARLEY

FILE 'BIOTECHDS'
L18       1924 BARLEY

FILE 'BIOSIS'
L19      44992 BARLEY

FILE 'EMBASE'
L20       3599 BARLEY

FILE 'HCAPLUS'
L21      46346 BARLEY

FILE 'NTIS'
L22       1236 BARLEY

FILE 'ESBIOBASE'
L23       6807 BARLEY

FILE 'BIOTECHNO'
L24       4377 BARLEY

FILE 'WPIDS'
L25       6458 BARLEY

FILE 'CABA'
L26      68117 BARLEY

FILE 'AGRICOLA'
L27      29207 BARLEY

TOTAL FOR ALL FILES
L28     254845 BARLEY

=> s glucanase#
FILE 'MEDLINE'
L29       1504 GLUCANASE#

FILE 'SCISEARCH'
L30       3351 GLUCANASE#

FILE 'LIFESCI'
L31       1567 GLUCANASE#

FILE 'BIOTECHDS'
L32       1262 GLUCANASE#

FILE 'BIOSIS'
L33       4293 GLUCANASE#

FILE 'EMBASE'
L34       1201 GLUCANASE#

FILE 'HCAPLUS'
L35       5819 GLUCANASE#

FILE 'NTIS'
L36         33 GLUCANASE#

FILE 'ESBIOBASE'
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L37       1223 GLUCANASE#

FILE 'BIOTECHNO'
L38       1283 GLUCANASE#

FILE 'WPIDS'
L39        760 GLUCANASE#

FILE 'CABA'
L40       2308 GLUCANASE#

FILE 'AGRICOLA'
L41       1553 GLUCANASE#

TOTAL FOR ALL FILES
L42      26157 GLUCANASE#

=> s psi or protein synthesis inhibit?
FILE 'MEDLINE'
          6550 PSI
       1284579 PROTEIN
        384281 SYNTHESIS
       1112008 INHIBIT?
          8063 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L43      14592 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'SCISEARCH'
         18926 PSI
       1100476 PROTEIN
        664316 SYNTHESIS
        900970 INHIBIT?
          3104 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L44      22022 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'LIFESCI'
          3242 PSI
        437217 "PROTEIN"
        101519 "SYNTHESIS"
        304854 INHIBIT?
          1496 PROTEIN SYNTHESIS INHIBIT?
                 ("PROTEIN"(W)"SYNTHESIS"(W)INHIBIT?)
L45       4730 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOTECHDS'
           705 PSI
        118090 PROTEIN
         20741 SYNTHESIS
         47772 INHIBIT?
            91 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L46        794 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOSIS'
          9149 PSI
       1398792 PROTEIN
        525769 SYNTHESIS
       1205900 INHIBIT?
          6128 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L47      15255 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'EMBASE'
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          6458 PSI
       1247513 "PROTEIN"
        501750 "SYNTHESIS"
       1000402 INHIBIT?
          7372 PROTEIN SYNTHESIS INHIBIT?
                 ("PROTEIN"(W)"SYNTHESIS"(W)INHIBIT?)
L48      13813 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'HCAPLUS'
         57715 PSI
       1638712 PROTEIN
       1128312 SYNTHESIS
       1665246 INHIBIT?
          5195 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L49      62897 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'NTIS'
          6927 PSI
         12416 PROTEIN
         34623 SYNTHESIS
         20259 INHIBIT?
            49 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L50       6976 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'ESBIOBASE'
          4497 PSI
        528929 PROTEIN
        138930 SYNTHESIS
        381375 INHIBIT?
          1639 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L51       6130 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'BIOTECHNO'
          3111 PSI
        623255 PROTEIN
        144368 SYNTHESIS
        301415 INHIBIT?
          3588 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L52       6691 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'WPIDS'
         12257 PSI
        116246 PROTEIN
         77225 SYNTHESIS
        218183 INHIBIT?
           137 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L53      12392 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'CABA'
          3762 PSI
        322393 PROTEIN
         76557 SYNTHESIS
        208614 INHIBIT?
           694 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L54       4455 PSI OR PROTEIN SYNTHESIS INHIBIT?

FILE 'AGRICOLA'
          1012 PSI
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        130587 PROTEIN
         38648 SYNTHESIS
         64720 INHIBIT?
           378 PROTEIN SYNTHESIS INHIBIT?
                 (PROTEIN(W)SYNTHESIS(W)INHIBIT?)
L55       1388 PSI OR PROTEIN SYNTHESIS INHIBIT?

TOTAL FOR ALL FILES
L56     172135 PSI OR PROTEIN SYNTHESIS INHIBIT?

=> s afp or antifungal protein
FILE 'MEDLINE'
          5619 AFP
         26521 ANTIFUNGAL
       1284579 PROTEIN
           112 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L57       5707 AFP OR ANTIFUNGAL PROTEIN

FILE 'SCISEARCH'
          3305 AFP
         15526 ANTIFUNGAL
       1100476 PROTEIN
           204 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L58       3481 AFP OR ANTIFUNGAL PROTEIN

FILE 'LIFESCI'
           756 AFP
          9461 "ANTIFUNGAL"
        437217 "PROTEIN"
            64 ANTIFUNGAL PROTEIN
                 ("ANTIFUNGAL"(W)"PROTEIN")
L59        806 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOTECHDS'
           175 AFP
          1181 ANTIFUNGAL
        118090 PROTEIN
            27 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L60        199 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOSIS'
          4951 AFP
         33206 ANTIFUNGAL
       1398792 PROTEIN
           195 ANTIFUNGAL PROTEIN
                 (ANTIFUNGAL(W)PROTEIN)
L61       5116 AFP OR ANTIFUNGAL PROTEIN

FILE 'EMBASE'
          4919 AFP
         23714 "ANTIFUNGAL"
       1247513 "PROTEIN"
            88 ANTIFUNGAL PROTEIN
                 ("ANTIFUNGAL"(W)"PROTEIN")
L62       4992 AFP OR ANTIFUNGAL PROTEIN

FILE 'HCAPLUS'
          3503 AFP
         23630 ANTIFUNGAL
       1638712 PROTEIN
           291 ANTIFUNGAL PROTEIN
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                (ANTIFUNGAL(W)PROTEIN)
L63        3750 AFP OR ANTIFUNGAL PROTEIN

FILE 'NTIS'
             83 AFP
            130 ANTIFUNGAL
          12416 PROTEIN
              0 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L64          83 AFP OR ANTIFUNGAL PROTEIN

FILE 'ESBIOBASE'
           1167 AFP
           4923 ANTIFUNGAL
         528929 PROTEIN
            120 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L65        1268 AFP OR ANTIFUNGAL PROTEIN

FILE 'BIOTECHNO'
           1356 AFP
           3941 ANTIFUNGAL
         623255 PROTEIN
             76 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L66        1413 AFP OR ANTIFUNGAL PROTEIN

FILE 'WPIDS'
            300 AFP
          10005 ANTIFUNGAL
         116246 PROTEIN
             24 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L67         323 AFP OR ANTIFUNGAL PROTEIN

FILE 'CABA'
            290 AFP
          24929 ANTIFUNGAL
         322393 PROTEIN
            119 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L68         390 AFP OR ANTIFUNGAL PROTEIN

FILE 'AGRICOLA'
             56 AFP
           3488 ANTIFUNGAL
         130587 PROTEIN
             52 ANTIFUNGAL PROTEIN
                (ANTIFUNGAL(W)PROTEIN)
L69          94 AFP OR ANTIFUNGAL PROTEIN

TOTAL FOR ALL FILES
L70       27622 AFP OR ANTIFUNGAL PROTEIN

=> s l14(8a)(serratia or marcescens)
FILE 'MEDLINE'
           6735 SERRATIA
           5214 MARCESCENS
L71          84 L1 (8A)(SERRATIA OR MARCESCENS)

FILE 'SCISEARCH'
           3908 SERRATIA
           3028 MARCESCENS
L72         104 L2 (8A)(SERRATIA OR MARCESCENS)
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FILE 'LIFESCI'
          2412 SERRATIA
          1791 MARCESCENS
L73         65 L3 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOTECHDS'
          1015 SERRATIA
           591 MARCESCENS
L74         74 L4 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOSIS'
          9223 SERRATIA
          6536 MARCESCENS
L75        130 L5 (8A)(SERRATIA OR MARCESCENS)

FILE 'EMBASE'
          6814 SERRATIA
          4635 MARCESCENS
L76         71 L6 (8A)(SERRATIA OR MARCESCENS)

FILE 'HCAPLUS'
          8134 SERRATIA
          6179 MARCESCENS
L77        199 L7 (8A)(SERRATIA OR MARCESCENS)

FILE 'NTIS'
           144 SERRATIA
           114 MARCESCENS
L78          2 L8 (8A)(SERRATIA OR MARCESCENS)

FILE 'ESBIOBASE'
           988 SERRATIA
           699 MARCESCENS
L79         58 L9 (8A)(SERRATIA OR MARCESCENS)

FILE 'BIOTECHNO'
          1615 SERRATIA
          1191 MARCESCENS
L80         60 L10(8A)(SERRATIA OR MARCESCENS)

FILE 'WPIDS'
          1217 SERRATIA
           515 MARCESCENS
L81         10 L11(8A)(SERRATIA OR MARCESCENS)

FILE 'CABA'
          1445 SERRATIA
           819 MARCESCENS
L82         57 L12(8A)(SERRATIA OR MARCESCENS)

FILE 'AGRICOLA'
           525 SERRATIA
           312 MARCESCENS
L83         28 L13(8A)(SERRATIA OR MARCESCENS)

TOTAL FOR ALL FILES
L84        942 L14(8A)(SERRATIA OR MARCESCENS)

=> s l14(8a)l28
FILE 'MEDLINE'
L85         21 L1 (8A)L15

FILE 'SCISEARCH'
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L86          48 L2 (8A)L16

FILE 'LIFESCI'
L87          17 L3 (8A)L17

FILE 'BIOTECHDS'
L88           7 L4 (8A)L18

FILE 'BIOSIS'
L89          54 L5 (8A)L19

FILE 'EMBASE'
L90          11 L6 (8A)L20

FILE 'HCAPLUS'
L91          80 L7 (8A)L21

FILE 'NTIS'
L92           1 L8 (8A)L22

FILE 'ESBIOBASE'
L93          21 L9 (8A)L23

FILE 'BIOTECHNO'
L94          17 L10(8A)L24

FILE 'WPIDS'
L95           2 L11(8A)L25

FILE 'CABA'
L96          45 L12(8A)L26

FILE 'AGRICOLA'
L97          15 L13(8A)L27

TOTAL FOR ALL FILES
L98         339 L14(8A) L28

=> s l42(8a)l28
FILE 'MEDLINE'
L99          71 L29(8A)L15

FILE 'SCISEARCH'
L100        173 L30(8A)L16

FILE 'LIFESCI'
L101         35 L31(8A)L17

FILE 'BIOTECHDS'
L102         34 L32(8A)L18

FILE 'BIOSIS'
L103        233 L33(8A)L19

FILE 'EMBASE'
L104         38 L34(8A)L20

FILE 'HCAPLUS'
L105        384 L35(8A)L21

FILE 'NTIS'
L106          0 L36(8A)L22

FILE 'ESBIOBASE'
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L107        47 L37(8A)L23

FILE 'BIOTECHNO'
L108        53 L38(8A)L24

FILE 'WPIDS'
L109        14 L39(8A)L25

FILE 'CABA'
L110       211 L40(8A)L26

FILE 'AGRICOLA'
L111        88 L41(8A)L27

TOTAL FOR ALL FILES
L112      1381 L42(8A) L28

=> s l56(8a)l28
FILE 'MEDLINE'
L113        22 L43(8A)L15

FILE 'SCISEARCH'
L114        33 L44(8A)L16

FILE 'LIFESCI'
L115        14 L45(8A)L17

FILE 'BIOTECHDS'
L116         3 L46(8A)L18

FILE 'BIOSIS'
L117        49 L47(8A)L19

FILE 'EMBASE'
L118        10 L48(8A)L20

FILE 'HCAPLUS'
L119        63 L49(8A)L21

FILE 'NTIS'
L120         0 L50(8A)L22

FILE 'ESBIOBASE'
L121        10 L51(8A)L23

FILE 'BIOTECHNO'
L122        15 L52(8A)L24

FILE 'WPIDS'
L123         0 L53(8A)L25

FILE 'CABA'
L124        61 L54(8A)L26

FILE 'AGRICOLA'
L125        17 L55(8A)L27

TOTAL FOR ALL FILES
L126       297 L56(8A) L28

=> s l70(8a)(aspergillus or giganteus)
FILE 'MEDLINE'
         22704 ASPERGILLUS
           235 GIGANTEUS
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L127        24 L57(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'SCISEARCH'
         23555 ASPERGILLUS
           824 GIGANTEUS
L128        27 L58(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'LIFESCI'
         12230 ASPERGILLUS
           331 GIGANTEUS
L129        13 L59(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOTECHDS'
          9004 ASPERGILLUS
            47 GIGANTEUS
L130         5 L60(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOSIS'
         39444 ASPERGILLUS
          1753 GIGANTEUS
L131        25 L61(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'EMBASE'
         17980 ASPERGILLUS
           184 GIGANTEUS
L132        17 L62(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'HCAPLUS'
         44345 ASPERGILLUS
           660 GIGANTEUS
L133        30 L63(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'NTIS'
           219 ASPERGILLUS
            30 GIGANTEUS
L134         0 L64(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'ESBIOBASE'
          6886 ASPERGILLUS
           229 GIGANTEUS
L135        19 L65(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'BIOTECHNO'
          7725 ASPERGILLUS
           121 GIGANTEUS
L136        19 L66(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'WPIDS'
          6421 ASPERGILLUS
            21 GIGANTEUS
L137         3 L67(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'CABA'
         25932 ASPERGILLUS
           659 GIGANTEUS
L138        17 L68(8A)(ASPERGILLUS OR GIGANTEUS)

FILE 'AGRICOLA'
         12258 ASPERGILLUS
           268 GIGANTEUS
L139        13 L69(8A)(ASPERGILLUS OR GIGANTEUS)

TOTAL FOR ALL FILES
L140       212 L70(8A)(ASPERGILLUS OR GIGANTEUS)
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=> s (l84 and (l98 or l112 or l126 or l140)) or (l98 and (l112 or l126 or l140)) or
(l112 and (l126 or l140)) or (l126 and l140)
FILE 'MEDLINE'
L141          2 (L71 AND (L85 OR L99 OR L113 OR L127)) OR (L85 AND (L99 OR L113
                OR L127)) OR (L99 AND (L113 OR L127)) OR (L113 AND L127)

FILE 'SCISEARCH'
L142         10 (L72 AND (L86 OR L100 OR L114 OR L128)) OR (L86 AND (L100 OR
                L114 OR L128)) OR (L100 AND (L114 OR L128)) OR (L114 AND L128)

FILE 'LIFESCI'
L143          4 (L73 AND (L87 OR L101 OR L115 OR L129)) OR (L87 AND (L101 OR
                L115 OR L129)) OR (L101 AND (L115 OR L129)) OR (L115 AND L129)

FILE 'BIOTECHDS'
L144          1 (L74 AND (L88 OR L102 OR L116 OR L130)) OR (L88 AND (L102 OR
                L116 OR L130)) OR (L102 AND (L116 OR L130)) OR (L116 AND L130)

FILE 'BIOSIS'
L145         10 (L75 AND (L89 OR L103 OR L117 OR L131)) OR (L89 AND (L103 OR
                L117 OR L131)) OR (L103 AND (L117 OR L131)) OR (L117 AND L131)

FILE 'EMBASE'
L146          1 (L76 AND (L90 OR L104 OR L118 OR L132)) OR (L90 AND (L104 OR
                L118 OR L132)) OR (L104 AND (L118 OR L132)) OR (L118 AND L132)

FILE 'HCAPLUS'
L147         18 (L77 AND (L91 OR L105 OR L119 OR L133)) OR (L91 AND (L105 OR
                L119 OR L133)) OR (L105 AND (L119 OR L133)) OR (L119 AND L133)

FILE 'NTIS'
L148          0 (L78 AND (L92 OR L106 OR L120 OR L134)) OR (L92 AND (L106 OR
                L120 OR L134)) OR (L106 AND (L120 OR L134)) OR (L120 AND L134)

FILE 'ESBIOBASE'
L149          5 (L79 AND (L93 OR L107 OR L121 OR L135)) OR (L93 AND (L107 OR
                L121 OR L135)) OR (L107 AND (L121 OR L135)) OR (L121 AND L135)

FILE 'BIOTECHNO'
L150          4 (L80 AND (L94 OR L108 OR L122 OR L136)) OR (L94 AND (L108 OR
                L122 OR L136)) OR (L108 AND (L122 OR L136)) OR (L122 AND L136)

FILE 'WPIDS'
L151          2 (L81 AND (L95 OR L109 OR L123 OR L137)) OR (L95 AND (L109 OR
                L123 OR L137)) OR (L109 AND (L123 OR L137)) OR (L123 AND L137)

FILE 'CABA'
L152         10 (L82 AND (L96 OR L110 OR L124 OR L138)) OR (L96 AND (L110 OR
                L124 OR L138)) OR (L110 AND (L124 OR L138)) OR (L124 AND L138)

FILE 'AGRICOLA'
L153          1 (L83 AND (L97 OR L111 OR L125 OR L139)) OR (L97 AND (L111 OR
                L125 OR L139)) OR (L111 AND (L125 OR L139)) OR (L125 AND L139)

TOTAL FOR ALL FILES
L154         68 (L84 AND (L98 OR L112 OR L126 OR L140)) OR (L98 AND (L112 OR
                L126 OR L140)) OR (L112 AND (L126 OR L140)) OR (L126 AND L140)

=> s (l14 and (l42 or l56 or l70)) or (l42 and (l56 or l70)) or (l56 and l70)
FILE 'MEDLINE'
L155        200 (L1 AND (L29 OR L43 OR L57)) OR (L29 AND (L43 OR L57)) OR (L43
                AND L57)
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FILE 'SCISEARCH'
L156       775 (L2 AND (L30 OR L44 OR L58)) OR (L30 AND (L44 OR L58)) OR (L44
               AND L58)

FILE 'LIFESCI'
L157       271 (L3 AND (L31 OR L45 OR L59)) OR (L31 AND (L45 OR L59)) OR (L45
               AND L59)

FILE 'BIOTECHDS'
L158       163 (L4 AND (L32 OR L46 OR L60)) OR (L32 AND (L46 OR L60)) OR (L46
               AND L60)

FILE 'BIOSIS'
L159       836 (L5 AND (L33 OR L47 OR L61)) OR (L33 AND (L47 OR L61)) OR (L47
               AND L61)

FILE 'EMBASE'
L160       128 (L6 AND (L34 OR L48 OR L62)) OR (L34 AND (L48 OR L62)) OR (L48
               AND L62)

FILE 'HCAPLUS'
L161       926 (L7 AND (L35 OR L49 OR L63)) OR (L35 AND (L49 OR L63)) OR (L49
               AND L63)

FILE 'NTIS'
L162         2 (L8 AND (L36 OR L50 OR L64)) OR (L36 AND (L50 OR L64)) OR (L50
               AND L64)

FILE 'ESBIOBASE'
L163       298 (L9 AND (L37 OR L51 OR L65)) OR (L37 AND (L51 OR L65)) OR (L51
               AND L65)

FILE 'BIOTECHNO'
L164       237 (L10 AND (L38 OR L52 OR L66)) OR (L38 AND (L52 OR L66)) OR (L52
               AND L66)

FILE 'WPIDS'
L165       133 (L11 AND (L39 OR L53 OR L67)) OR (L39 AND (L53 OR L67)) OR (L53
               AND L67)

FILE 'CABA'
L166       642 (L12 AND (L40 OR L54 OR L68)) OR (L40 AND (L54 OR L68)) OR (L54
               AND L68)

FILE 'AGRICOLA'
L167       325 (L13 AND (L41 OR L55 OR L69)) OR (L41 AND (L55 OR L69)) OR (L55
               AND L69)

TOTAL FOR ALL FILES
L168      4936 (L14 AND (L42 OR L56 OR L70)) OR (L42 AND (L56 OR L70)) OR (L56
               AND L70)

=> s l168 and synerg?
FILE 'MEDLINE'
         78101 SYNERG?
L169         9 L155 AND SYNERG?

FILE 'SCISEARCH'
         50986 SYNERG?
L170        22 L156 AND SYNERG?

FILE 'LIFESCI'
         18317 SYNERG?
L171        13 L157 AND SYNERG?
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FILE 'BIOTECHDS'
         1498 SYNERG?
L172       13 L158 AND SYNERG?

FILE 'BIOSIS'
        57949 SYNERG?
L173       23 L159 AND SYNERG?

FILE 'EMBASE'
        45152 SYNERG?
L174        6 L160 AND SYNERG?

FILE 'HCAPLUS'
        92509 SYNERG?
L175       37 L161 AND SYNERG?

FILE 'NTIS'
         3382 SYNERG?
L176        0 L162 AND SYNERG?

FILE 'ESBIOBASE'
        19238 SYNERG?
L177        9 L163 AND SYNERG?

FILE 'BIOTECHNO'
        16242 SYNERG?
L178        9 L164 AND SYNERG?

FILE 'WPIDS'
        21973 SYNERG?
L179        7 L165 AND SYNERG?

FILE 'CABA'
        14553 SYNERG?
L180       16 L166 AND SYNERG?

FILE 'AGRICOLA'
         4629 SYNERG?
L181       11 L167 AND SYNERG?

TOTAL FOR ALL FILES
L182      175 L168 AND SYNERG?

=> s l168 and transgen?
FILE 'MEDLINE'
        51532 TRANSGEN?
L183       21 L155 AND TRANSGEN?

FILE 'SCISEARCH'
        74581 TRANSGEN?
L184      146 L156 AND TRANSGEN?

FILE 'LIFESCI'
        27235 TRANSGEN?
L185       29 L157 AND TRANSGEN?

FILE 'BIOTECHDS'
        26418 TRANSGEN?
L186       77 L158 AND TRANSGEN?

FILE 'BIOSIS'
        69945 TRANSGEN?
L187       70 L159 AND TRANSGEN?
```

```
FILE 'EMBASE'
        40568 TRANSGEN?
L188       10 L160 AND TRANSGEN?

FILE 'HCAPLUS'
        68137 TRANSGEN?
L189      114 L161 AND TRANSGEN?

FILE 'NTIS'
          682 TRANSGEN?
L190        0 L162 AND TRANSGEN?

FILE 'ESBIOBASE'
        35052 TRANSGEN?
L191       30 L163 AND TRANSGEN?

FILE 'BIOTECHNO'
        34613 TRANSGEN?
L192       30 L164 AND TRANSGEN?

FILE 'WPIDS'
        12784 TRANSGEN?
L193       35 L165 AND TRANSGEN?

FILE 'CABA'
        26302 TRANSGEN?
L194       67 L166 AND TRANSGEN?

FILE 'AGRICOLA'
        14237 TRANSGEN?
L195       35 L167 AND TRANSGEN?

TOTAL FOR ALL FILES
L196      664 L168 AND TRANSGEN?

=> s (l154 or l182 or l196) not 1994-1999/py
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L197       21 (L141 OR L169 OR L183) NOT 1994-1999/PY

FILE 'SCISEARCH'
      5422124 1994-1999/PY
L198       84 (L142 OR L170 OR L184) NOT 1994-1999/PY

FILE 'LIFESCI'
       663072 1994-1999/PY
L199       20 (L143 OR L171 OR L185) NOT 1994-1999/PY

FILE 'BIOTECHDS'
        87969 1994-1999/PY
L200       47 (L144 OR L172 OR L186) NOT 1994-1999/PY

FILE 'BIOSIS'
      3354176 1994-1999/PY
L201       55 (L145 OR L173 OR L187) NOT 1994-1999/PY

FILE 'EMBASE'
      2370245 1994-1999/PY
L202        8 (L146 OR L174 OR L188) NOT 1994-1999/PY

FILE 'HCAPLUS'
      4675238 1994-1999/PY
L203       91 (L147 OR L175 OR L189) NOT 1994-1999/PY
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FILE 'NTIS'
       217918 1994-1999/PY
L204        0 (L148 OR L176 OR L190) NOT 1994-1999/PY

FILE 'ESBIOBASE'
      1329810 1994-1999/PY
L205       22 (L149 OR L177 OR L191) NOT 1994-1999/PY

FILE 'BIOTECHNO'
       643038 1994-1999/PY
L206       20 (L150 OR L178 OR L192) NOT 1994-1999/PY

FILE 'WPIDS'
      3745711 1994-1999/PY
L207       23 (L151 OR L179 OR L193) NOT 1994-1999/PY

FILE 'CABA'
       955245 1994-1999/PY
L208       53 (L152 OR L180 OR L194) NOT 1994-1999/PY

FILE 'AGRICOLA'
       441119 1994-1999/PY
L209       18 (L153 OR L181 OR L195) NOT 1994-1999/PY

TOTAL FOR ALL FILES
L210      462 (L154 OR L182 OR L196) NOT 1994-1999/PY

=> s l210 not 2000-2004/py
FILE 'MEDLINE'
      2375366 2000-2004/PY
L211        5 L197 NOT 2000-2004/PY

FILE 'SCISEARCH'
      4471553 2000-2004/PY
L212       32 L198 NOT 2000-2004/PY

FILE 'LIFESCI'
       450968 2000-2004/PY
L213        5 L199 NOT 2000-2004/PY

FILE 'BIOTECHDS'
        88917 2000-2004/PY
L214       16 L200 NOT 2000-2004/PY

FILE 'BIOSIS'
      2403721 2000-2004/PY
L215       17 L201 NOT 2000-2004/PY

FILE 'EMBASE'
      2055012 2000-2004/PY
L216        2 L202 NOT 2000-2004/PY

FILE 'HCAPLUS'
      4418551 2000-2004/PY
L217       20 L203 NOT 2000-2004/PY

FILE 'NTIS'
        71873 2000-2004/PY
L218        0 L204 NOT 2000-2004/PY

FILE 'ESBIOBASE'
      1296989 2000-2004/PY
L219        0 L205 NOT 2000-2004/PY
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FILE 'BIOTECHNO'
491187 2000-2004/PY
L220 1 L206 NOT 2000-2004/PY

FILE 'WPIDS'
3995175 2000-2004/PY
L221 0 L207 NOT 2000-2004/PY

FILE 'CABA'
728066 2000-2004/PY
L222 21 L208 NOT 2000-2004/PY

FILE 'AGRICOLA'
215807 2000-2004/PY
L223 6 L209 NOT 2000-2004/PY

TOTAL FOR ALL FILES
L224 125 L210 NOT 2000-2004/PY

=> d tot l223

L223 ANSWER 1 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI NopL, an effector protein of Rhizobium sp. NGR234, thwarts activation of plant defense reactions.
SO Plant physiology, p. 871-879
ISSN: 0032-0889
AU Bartsev, A.V.; Deakin, W.J.; Boukli, N.M.; McAlvin, C.B.; Stacey, G.; Malnoe, P.; Broughton, W.J.; Staehelin, C.
AN 2004:20225 AGRICOLA

L223 ANSWER 2 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI Only specific tobacco (Nicotiana tabacum) **chitinases** and beta-1,3-**glucanases** exhibit antifungal activity.
SO Plant physiology, Mar 1993. Vol. 101, No. 3. p. 857-863
Publisher: Rockville, MD : American Society of Plant Physiologists, 1926-
CODEN: PLPHAY; ISSN: 0032-0889
AU Sela-Buurlage, M.B.; Ponstein, A.S.; Bres-Vloemans, S.A.; Melchers, L.S.; Elzen, P.J.M. van den.; Cornelissen, B.J.C.
AN 94:4050 AGRICOLA

L223 ANSWER 3 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI Extracellular targeting of the vacuolar tobacco proteins AP24, **chitinase** and beta-1,3-**glucanase** in **transgenic** plants.
SO Plant molecular biology : an international journal on molecular biology, biochemistry and genetic engineering, Feb 1993. Vol. 21, No. 4. p. 583-593
Publisher: Dordrecht : Kluwer Academic Publishers.
ISSN: 0167-4412
AU Melchers, L.S.; Sela-Buurlage, M.B.; Vloemans, A.S.; Woloshuk, C.P.; Roekel, J.S.C. van; Pen, J.; Elzen, P.J.M. van den; Cornelissen, B.J.C.
AN 93:42465 AGRICOLA

L223 ANSWER 4 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States

of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI The function of vacuolar beta-1,3-**glucanase** investigated by antisense transformation. Susceptibility of **transgenic** Nicotiana sylvestris plants to Cercospora nicotianae infection.
SO Plant molecular biology : an international journal on molecular biology, biochemistry and genetic engineering, Aug 1992. Vol. 19, No. 5. p. 803-813
Publisher: Dordrecht : Kluwer Academic Publishers.
ISSN: 0167-4412
AU Neuhaus, J.M.; Flores, S.; Keefe, D.; Ahl-Goy, P.; Meins, F. Jr
AN 92:112708 AGRICOLA

L223 ANSWER 5 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI High-level expression of a tobacco **chitinase** gene in Nicotiana sylvestris. Susceptibility of **transgenic** plants to Cercospora nicotianae infection.
SO Plant molecular biology : an international journal on fundamental research and genetic engineering, Jan 1991. Vol. 16, No. 1. p. 141-151 ill
Publisher: Dordrecht : Kluwer Academic Publishers.
ISSN: 0167-4412
AU Neuhaus, J.M.; Ahl-Goy, P.; Hinz, U.; Flores, S.; Meins, F.
AN 91:31208 AGRICOLA

L223 ANSWER 6 OF 6 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States of America. It contains copyrighted materials. All rights reserved. (2004) on STN
TI Biochemical and molecular characterization of three barley seed proteins with antifungal properties.
SO The Journal of biological chemistry, Jan 25, 1991. Vol. 266, No. 3. p. 1564-1573 ill
Publisher: Baltimore, Md. : American Society for Biochemistry and Molecular Biology.
CODEN: JBCHA3; ISSN: 0021-9258
AU Leah, R.; Tommerup, H.; Svendsen, I.; Mundy, J.
AN 91:31079 AGRICOLA

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	53.15	53.57

STN INTERNATIONAL LOGOFF AT 10:00:37 ON 07 JUL 2004

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2472	chitinase$1	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:04
L2	17231	barley	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:04
L3	2953	glucanase$1	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:04
L4	166890	psi or protein synthesis inhibit$8	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:05
L5	2030	afp or antifungal protein	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:05
L6	94	1 near6 (serratia or marcesens)	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:06
L7	42	1 near6 2	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:06
L8	119	3 near6 2	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:06
L9	16	4 near6 2	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:06
L10	27	5 near6 (aspergillus or giganteus)	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:07
L11	34	(6 and (7 or 8 or 9 or 10)) or (7 and (8 or 9 or 10)) or (8 and (9 or 10)) or (9 and 10)	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:08
L12	13	(6 or 7 or 8 or 9 or 10) same synerg$	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:09
L13	36	(6 or 7 or 8 or 9 or 10) same transgen$	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:10
L14	1207	(1 near10 (3 or 4 or 5)) or (3 near10 (4 or 5)) or (4 near10 5)	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:11
L15	25	14 near10 synerg$	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:11
L16	52	14 near10 transgen$	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:11
L17	104	11 or 12 or 13 or 15 or 16	US-PGPUB; USPAT	ADJ	OFF	2004/07/07 09:12

PGPUB-DOCUMENT-NUMBER: 20040093646

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040093646 A1

TITLE: Production of lysosomal enzymes in plants by transient expression

PUBLICATION-DATE: May 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Erwin, Robert L.	Davis	CA	US	
Grill, Laurence K.	Vacaville	CA	US	
Pogue, Gregory P.	Vacaville	CA	US	
Turpen, Thomas H.	Vacaville	CA	US	
Kumagai, Monto H.	Kailua	HI	US	

APPL-NO: 10/ 684349

DATE FILED: October 9, 2003

RELATED-US-APPL-DATA:

child 10684349 A1 20031009

parent continuation-of 09626127 20000726 US PENDING

child 09626127 20000726 US

parent continuation-in-part-of 09316572 19990521 US ABANDONED

child 09316572 19990521 US

parent continuation-of 08324003 19941014 US GRANTED

parent-patent 5977438 US

child 08324003 19941014 US

parent continuation-in-part-of 08176414 19931229 US GRANTED

parent-patent 5811653 US

child 08176414 19931229 US

parent continuation-in-part-of 07997733 19921230 US ABANDONED

child 08324003

parent continuation-in-part-of 08184237 19940119 US GRANTED

parent-patent 5589367 US

child 08184237 19940119 US

parent continuation-in-part-of 07923692 19920731 US GRANTED

parent-patent 5316931 US

child 07923692 19920731 US

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07641617 19910116 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07737899 19910726 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07739143 19910801 US ABANDONED

child 07739143 19910801 US

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07600244 19901022 US

parent continuation-of 07310881 19890217 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160766 19880226 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160771 19880226 US ABANDONED

child 07641617

parent continuation-of 07347637 19890505 US ABANDONED

child 07737899

parent continuation-of 07363138 19890608 US ABANDONED

child 07363138 19890608 US

parent continuation-in-part-of 07219279 19880715 US ABANDONED

child 07739143

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07739143

parent continuation-in-part-of 07641617 19910116 US ABANDONED

child 07739143

parent continuation-in-part-of 07737899 19910726 US ABANDONED

US-CL-CURRENT: 800/288, 435/206 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to the production of enzymatically active recombinant human and animal lysosomal enzymes involving construction and expression of recombinant expression constructs comprising coding sequences of human or animal lysosomal enzymes in a plant expression system. The plant expression system provides for post-translational modification and processing to produce a recombinant gene product exhibiting enzymatic activity. The invention is demonstrated by working examples in which transgenic tobacco plants express recombinant expression constructs comprising human glucocerebrosidase nucleotide sequences. The invention is also demonstrated by working examples in which transfected tobacco plants express recombinant viral expression constructs comprising human .alpha. galactosidase nucleotide sequences. The recombinant lysosomal enzymes produced in accordance with the invention may be used for a variety of purposes, including but not limited to enzyme replacement therapy for the therapeutic treatment of human and animal lysosomal storage diseases.

PRIORITY DATA

[0001] The present application is a division of application Ser. No. 09/626,127, filed Jul. 26, 2000, which is a continuation-in-part of application Ser. No. 09/316,572, filed May 21, 1999, now abandoned, which is a continuation of application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438, which is a continuation-in-part of application Ser. No. 08/176,414, filed on Dec. 29, 1993, now U.S. Pat. No. 5,811,653, which is a continuation-in-part of application Ser. No. 07/997,733, filed Dec. 30, 1992, now abandoned. Application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438 is also a continuation-in-part of application Ser. No. 08/184,237, filed Jan. 19, 1994, now U.S. Pat. No. 5,589,367, which is a continuation-in-part of application Ser. No. 07/923,692, filed Jul. 31, 1992, now U.S. Pat. No. 5,316,931, which is a continuation-in-part of application Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, application Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned, and application Ser. No. 07/739,143, filed Aug. 1, 1991, now abandoned. Application Ser. No. 07/600,244 is a continuation of application Ser. No. 07/310,881, filed Feb. 17, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/160,766 and Ser. No. 07/160,771, both filed on Feb. 26, 1988 and now abandoned. Application Ser. No. 07/641,617 is a continuation of application Ser. No. 07/347,637, filed May 5, 1989, now abandoned. Application Ser. No. 07/737,899 is a continuation of application Ser. No. 07/363,138, filed Jun. 8, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/219,279, filed Jul. 15, 1988, now abandoned. Application Ser. No. 07/739,143 is a continuation-in-part of application Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, and Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned. All of the above referenced priority applications are incorporated herein by reference in their entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (13):
[0063] Plant proteins do not require N-linked oligosaccharides for correct sorting into vacuoles (35,37,38). Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a

CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48). Sorting of Gal-A to the lysosome is likely to occur by the well-characterized mannose-6-phosphate receptor pathway in mammalian cells. We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi network. This signal appears to function in plant cells, presumably for vacuolar localization.

Detail Description Paragraph - DETX (311):

[0328] 46. Melchers, L. S., Sela-Buurlage, M. B., Vloemans, S. A., Woloshuk, C. P., Van Roekel, J. S. C., Pen, J., Van den Elzen, P. J. M., Comelissen, B. J. C. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and .beta.-1,3-glucanase in transgenic plants. Plant Mol. Biol. 21:583, 1993.

PGPUB-DOCUMENT-NUMBER: 20040093640

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040093640 A1

TITLE: Fungal resistant transgenic plants

PUBLICATION-DATE: May 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chen, Zhizheng	Fort Collins	CO	US	
Hallier, Sonia	Livermore	CO	US	
Deng, Xinmin	Camrose		CA	

APPL-NO: 10/ 470427

DATE FILED: December 15, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
US	60264776	2001US-60264776	January 29, 2001

PCT-DATA:
APPL-NO: PCT/US02/02444
DATE-FILED: Jan 29, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 800/284, 800/279

ABSTRACT:

Transgenic plants that are resistant to Sclerotinia and Phoma lingam are described as well as methods for producing such transgenic plants.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (12):

[0009] The transgenic plant can include three nucleic acid constructs, wherein each construct includes a regulatory element operably linked to a nucleic acid encoding one of the polypeptides. Chitinase, P-1,3-glucanase, and osmotin polypeptides can be constitutively expressed in the stem or leaves of the transgenic plant. The transgenic plant can be resistant to Sclerotinia on the stem or on the leaves.

Detail Description Paragraph - DETX (7):

[0025] Expressing combinations of chitinase, .beta.-1,3-glucanase, osmotin (e.g., AP24), and PR1 provides resistance to sclerotinia and/or blackleg in transgenic plants of the invention. For example, expressing AP24 and chitinase in Brassica provides enhanced resistance to blackleg relative to a control plant that does not express the exogenous polypeptides. Expressing chitinase, glucanase, and AP24 provides enhanced resistance to S. sclerotiorum relative to

a control plant that does not express the exogenous polypeptides. In some embodiments, two or more chitinases can be expressed with AP24 or in combination with AP24 and glucanase. For example, a barley class II chitinase and a tobacco class I chitinase can be expressed in combination with other host defense polypeptides. Two or more glucanases also can be expressed in combination with other host defense polypeptides. For example, class I and class II glucanases can be expressed in combination with other host defense polypeptides.

Detail Description Paragraph - DETX (75):

[0084] There appeared to be a correlation between glucanase and chitinase expression in leaves among the 18 tested lines (FIG. 3), indicating that the transgenes from the same transformation event were regulated similarly. R.sup.2=0.6 for all 18 lines; R.sup.2=0.7 when lines 158 and 87 were excluded. Given the apparent correlation between glucanase and chitinase expression, the expression values for these two proteins were grouped together in the form of a glucanase-chitinase average relative to the 213 line to increase the accuracy of the intrinsic value of each line for glucanase and chitinase expression. The 158 and 87 lines had high glucanase expression without any chitinase expression (for line 158, chi=72 and glu=0, for the 87 line, chi=49 and glu=0).

Detail Description Paragraph - DETX (76):

[0085] An apparent correlation also was observed between glucanase and chitinase expression in the stem. When lines 98, 117, 158 and 220 were excluded, R.sup.2=0.9, indicating that regulation of the glucanase and chitinase transgenes in the stem was similar to that of the leaves.

Detail Description Paragraph - DETX (80):

[0087] Expression levels of chitinase and glucanase were measured in infected leaves to determine if transgenes were differently regulated during infection and to determine if endogenous chitinases and glucanases were expressed during infection. The study was carried out by ELISA using detached leaves that were subjected to the sclerotinia disease test in vitro. Two plants from each line were evaluated, using four separate ELISA reactions and two wells per sample.

Claims Text - CLTX (15):

14. The transgenic plant of claim 1, wherein said chitinase, .beta.-1,3-glucanase, and osmotin polypeptides are constitutively expressed in the stem of said transgenic plant.

Claims Text - CLTX (16):

15. The transgenic plant of claim 1, wherein said chitinase, .beta.-1,3-glucanase, and osmotin polypeptides are constitutively expressed in leaves of said transgenic plant.

PGPUB-DOCUMENT-NUMBER: 20040078842

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040078842 A1

TITLE: Chitinases, derived from carnivorous plants polynucleotide sequences encoding thereof, and methods of isolating and using same

PUBLICATION-DATE: April 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Zilberstein, Aviah	Holon		IL	
Eilenberg, Haviva	Ramat Hasharon		IL	
Schuster, Silvia	Raanana		IL	

APPL-NO: 10/ 451794

DATE FILED: November 24, 2003

PCT-DATA:
APPL-NO: PCT/IL02/00044
DATE-FILED: Jan 17, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 800/279, 424/94.61 , 435/200

ABSTRACT:

The present invention provides an enzymatic composition comprising at least one protein isolated from a tissue or soup of a carnivorous plant, the at least one protein being characterized with an endo-chitinase activity.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (25):

[0090] FIG. 22 illustrates a Western blot demonstrating the expression and correct processing of Nkchit1b-gI-HA fusion protein in transgenic tobacco plants. Extracts of leaf tissue (0.5 g) from six transgenic plants (lanes 1-6) produced by Agrobacterium-mediated transformation of tobacco leaf discs with pPCV702-chit1-HA, as described in the Examples section, a wild type plant (negative control, lane NN) and a transgenic Serratia chitinase-HA expressing plant (positive control, lane 7) were prepared as detailed in Methods, and separated on a 12% SDS-PAGE gel. Proteins were blotted onto PVDF membrane, and fusion proteins were detected via probing with polyclonal rat anti-HA antibodies and visualization with Alkaline Phosphatase-conjugated affinity purified Goat anti-rat IgG (black bands). Note the presence of a 36 kDa band representing varying levels of expression of novel Nepenthes chitinase fusion protein (arrowhead, lanes 3, 4 and 5), and the positive identification of 59 kDa Serratia chitinase-HA fusion protein (lane 7).

Brief Description of Drawings Paragraph - DRTX (26):

[0091] FIG. 23 illustrates a Western blot demonstrating the expression and correct processing of Nkchit2b-gII-HA fusion protein in transgenic tobacco plants. Extracts of leaf tissue (0.5 g) from five transgenic plants (lanes 1-5) produced by Agrobacterium-mediated transformation of tobacco leaf discs with pPCV702-chit2-HA, as described in the Examples section, a wild type plant (negative control, lane NN) and a transgenic Serratia chitinase-HA expressing plant (positive control, lane 6) were prepared and separated as detailed in FIG. 22 above. Blotting and detection of proteins was performed as detailed in FIG. 22. Note the presence of a 32.7 kDa band representing varying levels of expression of novel Nepenthes chitinase fusion protein (arrowhead, lanes 2 and 4), and the positive identification of 59 kDa Serratia chitinase-HA fusion protein (lane 6).

Brief Description of Drawings Paragraph - DRTX (29):

[0094] FIGS. 26a-b illustrate protein quantification evaluation of Serratia marcescens chitinase and Nepenthes trap soup chitinase. The indicated proteins were resolved by gel electrophoresis and visualized by silver staining. FIG. 26a shows the indicated volumes of commercially available Serratia marcescens (58 kDa) and specified amounts of bovine serum albumine (BSA, 66 kDa). FIG. 26b shows the indicated volumes of Nepenthes trap soup chitinase and specified amounts of carbonic anhydrase (29 kDa). SM--indicates molecular weight marker.

Brief Description of Drawings Paragraph - DRTX (30):

[0095] FIGS. 27a-b depicts the results of chitinase activity assay performed with Serratia marcescens chitinase and Nepenthes trap soup chitinase. Chitinase activity was determined for 100 ng of commercial Serratia marcescens chitinase (FIG. 27a) and for 20-30 ng of trap soup chitinase (FIG. 27b). The tetramer p-nitrophenyl-.beta.-D-N-N'-N"-triacet- ylchitotriose was used as a substrate and p-nitrophenyl release was determined.

Detail Description Paragraph - DETX (23):

[0117] Km--the proteins of the present invention may be considered as having high Km values as compared to Serratia marcescens chitinase, however it is presumed that these polypeptides apparently don't follow the Michaelis-Menten model, as a sigmoidal plot of reaction velocity versus substrate concentration is observed, suggesting that the polypeptides of the present invention are allosteric enzymes. This is substantiated by the observations that chitin induction significantly increases the enzymatic activity of the polypeptides and also by the observation that the enzymes of the present invention may include more than one subunit (see Example 20 of the Examples section).

Detail Description Paragraph - DETX (25):

[0119] Antibody reactivity--Unlike trap-tissue and leaf derived chitinase proteins, the trap-soup chitinase proteins of the present invention are not reactive with a polyclonal antibody directed to Serratia marcescens chitinase (ChiAII), indicating that the proteins do not share antigenic epitopes with the Serratia marcescens chitinase (see Example 2 of the Examples section).

Detail Description Paragraph - DETX (29):

[0123] In contrast to Serratia chitinase, the anti-fungal activity of the enzymatic composition of the present invention is fungicidal and as such can be used for both agricultural and therapeutic purposes.

Detail Description Paragraph - DETX (175):

[0267] Western analysis. SDS-PAGE was performed with 12 or 15% resolving gel and 5% stacking gel. The protein was transferred onto PVDF membrane (Gelman) and the chitinase was detected using either rabbit polyclonal antibodies against Serratia marcescens chitinase (ChiAII) (Jones et al., 1986) or rat anti-HA antibodies (in the case of transgenic expression in plants) [Rat monoclonal antibody (clone 3F10), Roch, Cat. No. 1867423] and then visualized by Alkaline Phosphatase-conjugated affinity purified goat anti-rabbit or anti-rat IgG (Affinipure Goat-anti-Rat, Jackson Immunoresearch Cat. No. 112-055-003), respectively.

Detail Description Paragraph - DETX (196):

[0284] Western blot analyses were performed and probed with polyclonal antibodies against Serratia marcescens chitinase (ChiAII) to reveal differences in the antigenic character of the Nepenthes chitinases extracted from the various tissues. FIG. 2A shows the western blot of a 15% SDS-PAGE gel loaded with concentrates of either trap(C) or leaf tissue(L) extract (50 .mu.l) and trap soup(S) (40 .mu.l). Although the anti-Serratia ChiAII antibodies recognized chitinases from both the trap and leaf tissue (marked by the lower arrow), they did not recognize the soup chitinase. The gel in FIG. 2B confirms the lack of antigenic identity between trap soup and leaf (L) or trap tissue chitinases, demonstrating no immune recognition even when the protein concentration of the trap soup sample(S) was increased 22 fold, representing 875 .mu.l initial trap soup volume.

Detail Description Paragraph - DETX (199):

[0285] Endochitinases hydrolytically degrade chitin within the polymer. Conversely, exochitinase digestion is restricted to degradation at the termini. Evaluation of endo- versus exo-chitinase activity of Nepenthes chitinases was carried out as follows: Soup as well as trap and leaf tissue extracts were tested for exochitinase and chitin 1,4-chitobiosidase activity using the substrates p-Nitrophenyl N-acetyl-D glucosaminide (dimer) or p-Nitrophenyl-D-N,N-diacetyl chitobiose (trimer), respectively. Chitinase activity was detected spectrophotometrically at 410 nm by measuring nitrophenol absorbance resulting from the hydrolysis of the above substrates at pH 6.5. None of the Nepenthes chitinases showed any exochitinase or chitobiosidase activity, while Serratia ChiAII chitinase exhibited chitobiosidase activity. Soup, trap and leaf Nepenthes chitinases all hydrolyze glycol-chitin (FIG. 1), indicating that all three chitinases are endochitinases.

Detail Description Paragraph - DETX (205):

[0287] To further differentiate between Nepenthes chitinases, samples of non-boiled and boiled (5 min) soup and leaf extract were separated on 15% SDS-PAGE in the presence of both SDS and 2-mercaptoethanol. The gels were then renatured as described in Example 4 and overlayed by a chitinase activity gel. FIGS. 4A and 4B clearly demonstrate that the addition of the reducing agent 2-mercaptoethanol, in addition to SDS, completely inactivates the soup chitinase without affecting the leaf chitinase activity. In contrast, Serratia chitinase activity was abolished only in the boiled sample. Thus the novel soup chitinase clearly differs from that of the leaves. Furthermore, the importance of intact S--S bonds for the trap soup chitinase activity indicates that the trap soup chitinase is a dimer held together by inter-chain disulphide bonds (also demonstrated by its relative migration on gels displayed in FIGS. 1 and 3). To date, most active forms of plant chitinases identified are monomers of approximately 25-40 kDa. Thus the identification of a dimeric chitinase is extremely rare and unexpected.

Detail Description Paragraph - DETX (220):

[0294] These results indicate that while normal trap soup had no effect, the chitin-induced trap soup was very efficient (1/8th dilution of the sample) in

inhibiting growth of Candida albicans. When the injected chitin was present in the assayed sample, the MIC increased. This indicates that chitinase, indeed, plays a crucial role in the antifungal activity. Moreover, when the minimal fungicidal concentration (MFC) for the chitin-induced soup was determined, it was found to be at 1/4 the dilution of the sample (FIG. 10a), indicating extensive disruption of the C. albicans at that concentration. Furthermore, while commercial Serratia marcescens chitinase had no inhibitory effect, all three additional carnivorous plants leaf extracts were very potent in inhibiting Candida albicans growth (Table V, see also FIG. 24).

Detail Description Paragraph - DETX (244):

[0306] To study the possible effect of the abovementioned unique amino acid sequences, a three-dimensional structural modeling (SWISS-MODEL Protein Modeling, http://www.expasy.ch/swissmod/SWISS-MODEL) of NkCHIT2b (which appears to be the constituent chitinase in the trap soup) was performed. FIGS. 16A and 16B summarize this information. The prediction is based on the crystal structure of endochitinase from Hordeum vulgare L. (barley) seeds (Song et al., 1993; PDB and Swissprot accession numbers are 1CNS and p23951, respectively). FIG. 16A shows a segment of a multiple sequence alignment of NkCHIT2b, with the sequences from the gene bank that show closest homology to NkCHIT2b, into which the sequence of barley chitinase was included. In addition to the above mentioned altered amino acid residues, two NkCHIT2b glutamic acids (#134 and 156) were marked, which have been shown to be essential for the catalytic activity in barley chitinase, as well as the asparagine #191 which in barley is located in the substrate binding cleft (Andersen et al., 1997). Mutation of either of these glutamic acids (Glu 67 and Glu 89 in barley chitinase, which correspond to Glu 134 and Glu 156 in NkCHIT2b) results in a substantial loss of barley chitinase activity. Similarly, the asparagine (Asn 124 in barley which corresponds to Asn 191 in NkCHIT2b), was shown to play an important functional role (Andersen et al., 1997).

Detail Description Paragraph - DETX (245):

[0307] FIG. 16A shows the model of NkCHIT2b when viewed from left and right side of the same molecule. The locations of the relevant amino acid residues are marked on the molecule and they are specified at the left and right side of the molecule according to their relative location. It can be seen that the Glu 134 and 156 and Asn 191, that have been shown to be crucial for catalytic activity in barley chitinase, are located in the catalytic cleft. It is interesting to note that the hydrophobic amino acid Phe 190 of NkCHIT2b replaces the highly conserved polar tyrosine located in the catalytic cleft, adjacent to the Asn 191, which in barley has an important functional role. Moreover, Glu 211 and Lys 212, which both are charged amino acids, replace hydrophobic (alanine) and polar (threonine) amino acids in the corresponding locations in barley. Such changes in charge in the vicinity of the catalytic cleft, could account for changes in catalytic activity unique to the novel Nepenthes chitinase.

Detail Description Paragraph - DETX (259):

[0315] Nkchit1b-gI, Nkchit2b-gII and Nkchit2b-cIII were synthesized by direct PCR strategy using specific proof reading Taq polymerase and specially designed primers (Table IV) that enable further cloning of the genes into a plasmid carrying a plant expression cassette with the HA encoding sequence. Thereafter, the chitinase-HA cassette was cloned into the pPCV702 binary vector [Koncz. et al. (1989) Proc. Natl. Acad. Sci. USA 86:8467-8471] for subsequent Agrobacterium-mediated transformation of tobacco leaf discs. FIG. 21 shows the final pPCV702 vector which in addition to the nptII selectable marker carries either the Nkchit1b-gI, Nkchit2b-gII or Nkchit2b-cIII gene fused to the sequence encoding HA epitope at the 3' end and driven by the constitutive CaMV 35S promoter. After co-cultivation of leaf discs with the

engineered Agrobacterium, shoot regeneration was induced in the presence of kanamycin and hormones. Kanamycin resistant plants obtained from the transformations were screened for the expression of each of the chitinase-HA fused proteins by Western analysis using anti-HA antibodies. FIGS. 22 and 23 show typical Western blot analyses of the kanamycin resistant plants. Wild type tobacco (NN) and transgenic tobacco expressing Serratia chitinase fused to the HA tag (MW.about.59,000 Dalton) were used as negative and positive controls, respectively. The anticipated sizes of chitinase1 and chitinase2 protein fused to the Ha tag are 36,000 and 32,700 Dalton, respectively. Four of the six plants screened expressed the chitinase1 enzyme (FIG. 22). The observed molecular weight was as expected, indicating accurate processing of the Nepenthes chitinase in the tobacco plants. Plant #4 showed a relatively high amount of chitinase1-HA product, indicating that this transgenic plant contains several copies of introduced chitinase1 transgene. Thus, plant #4 is a good candidate for subsequent purification of the chitinase1 protein. In FIG. 23 demonstrates the expression of the chitinase2 enzyme in two of the five plants screened. The observed molecular weight of the chitinase2-HA protein was 32,700 Daltons, indicating accurate intron splicing in these transgenic plants as well.

Detail Description Paragraph - DETX (267):
[0319] To further characterize biochemically the chitinase activity present in the Nepenthes trap soup, the kinetic properties of the soup chitinase was compared with that of recombinant Serratia marcescens chitinase.

Detail Description Paragraph - DETX (268):
[0320] Sterile trap soup was collected from closed traps and concentrated 4.8 fold by speedvaccing. Serratia marcescens chitinase (lyophilized powder) was purchased from Sigma (C1650) and dissolved in H.sub.2O.

Detail Description Paragraph - DETX (269):
[0321] Due to the very low amount of chitinase protein/s present in the trap soup, the exact protein amount could not be detected by the regular methods (see Example 6). Thus, to estimate the enzyme amount used for activity assays the amount of the .about.32-35 kDa band which corresponded to the deduced sizes of the isolated cDNAs of Nepenthes chitinase/s protein concentration was determined by SDS-PAGE and silver staining. Similarly, the amount of the commercial Serratia marcescens chitinase was also determined.

Detail Description Paragraph - DETX (270):
[0322] FIG. 1. shows the approximate quantitation of the Nepenthes and Serratia chitinases on 12% SDS-PAGE gels after silver staining. BSA (.about.66 kDa) and Carbonic anhydrase (29 kDa) were used for quantity calibration of Serratia (.about.58 kDa) and Nepenthes (.about.32-35 kDa) chitinases, respectively. The amount of chitinase used for the activity assays was estimated to be approximately 100 ng (in 5 .mu.l) for Serratia. In case of Nepenthes chitinase, there was only a slight band at 32-35 kDa which could represent the enzyme and therefore, the amount of chitinase was estimated to be maximally in the range of 20-30 ng (in 30 .mu.l).

Detail Description Paragraph - DETX (272):
[0324] FIG. 2. shows that the Nepenthes chitinase seems to have a higher K.sub.m than the Serratia enzyme, but its activity is still linearly correlated with substrate concentration, while Serratia chitinase reached maximal activity already at substrate concentration of 9 .mu.g/assay. Furthermore, under repeated assays the Nepenthes enzyme showed a relationship between Vo and [S] that differs from the normal Michaelis-Menten behavior (data not shown). A sigmoid (rather than hyperbolic) saturation curve, which is characteristic for allosteric enzymes, was observed. This sigmoid kinetic behavior generally

reflects cooperative interactions between multiple protein subunits [Lehninger et al., (1993) Principles in Biochemistry 229-233]. It has previously been shown that the presence of .beta.-mercaptoethanol, in addition to SDS, inactivates the soup chitinase while it does not affect leaf chitinase activity (Example 5.). These results already suggested that the trap soup chitinase might be a dimer held together by inter-molecular disulphide bonds. Although most plant chitinases are active as monomers of .about.25-35 kDa, a dimeric chitinase has been identified in the seeds of Job's tears (reviewed by L. S. Graham and M. B. Sticklen, 1994). A clear conclusion about the nature of the Nepenthes chitinase cannot be made since more than one type of chitinase might be present in the trap soup whose activity was studied above. Therefore, final characterization will be performed only with the purified enzymes, following their separate expression in transgenic plants.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040064855 A1

TITLE: Production of lysosomal enzymes in plants by transient expression

PUBLICATION-DATE: April 1, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Erwin, Robert L.	Davis	CA	US	
Grill, Laurence K.	Vacaville	CA	US	

APPL-NO: 10/ 684300

DATE FILED: October 9, 2003

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child 09316572 19990521 US

parent continuation-of 08324003 19941014 US GRANTED

parent-patent 5977438 US

child 08324003 19941014 US

parent continuation-in-part-of 08176414 19931229 US GRANTED

parent-patent 5811653 US

child 08176414 19931229 US

parent continuation-in-part-of 07997733 19921230 US ABANDONED

child 08324003

parent continuation-in-part-of 08184237 19940119 US GRANTED

parent-patent 5589367 US

child 08184237 19940119 US

parent continuation-of 07923692 19920731 US GRANTED

parent-patent 5316931 US

child 07923692 19920731 US

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07641617 19910116 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07737899 19910726 US ABANDONED

child 07923692 19920731 US

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parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07600244 19901022 US

parent continuation-of 07310881 19890217 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160766 19880226 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160771 19880226 US ABANDONED

child 07641617

parent continuation-of 07347637 19890505 US ABANDONED

child 07737899

parent continuation-of 07363138 19890608 US ABANDONED

child 07363138 19890608 US

parent continuation-in-part-of 07219279 19880715 US ABANDONED

child 07739143

parent continuation-in-part-of 07600244 19901022 US ABANDONED

US-CL-CURRENT: 800/288, 435/206 , 800/317.3

ABSTRACT:

The invention relates to the production of enzymatically active recombinant human and animal lysosomal enzymes involving construction and expression of recombinant expression constructs comprising coding sequences of human or animal lysosomal enzymes in a plant expression system. The plant expression

system provides for post-translational modification and processing to produce a recombinant gene product exhibiting enzymatic activity. The invention is demonstrated by working examples in which transgenic tobacco plants express recombinant expression constructs comprising human glucocerebrosidase nucleotide sequences. The invention is also demonstrated by working examples in which transfected tobacco plants express recombinant viral expression constructs comprising human .alpha. galactosidase nucleotide sequences. The recombinant lysosomal enzymes produced in accordance with the invention may be used for a variety of purposes, including but not limited to enzyme replacement therapy for the therapeutic treatment of human and animal lysosomal storage diseases.

PRIORITY DATA

[0001] The present application is a division of application Ser. No. 09/626,127, filed Jul. 26, 2000, which is a continuation-in-part of application Ser. No. 09/316,572, filed May 21, 1999, now abandoned, which is a continuation of application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438, which is a continuation-in-part of application Ser. No. 08/176,414, filed on Dec. 29, 1993, now U.S. Pat. No. 5,811,653, which is a continuation-in-part of application Ser. No. 07/997,733, filed Dec. 30, 1992, now abandoned. Application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438 is also a continuation-in-part of application Ser. No. 08/184,237, filed Jan. 19, 1994, now U.S. Pat. No. 5,589,367, which is a continuation-in-part of application Ser. No. 07/923,692, filed Jul. 31, 1992, now U.S. Pat. No. 5,316,931, which is a continuation-in-part of applications Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, application Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned, and application Ser. No. 07/739,143, filed Aug. 1, 1991, now abandoned. Application Ser. No. 07/600,244 is a continuation of application Ser. No. 07/310,881, filed Feb. 17, 1989, now abandoned, which is a continuation-in-part of applications Ser. No. 07/160,766 and Ser. No. 07/160,771, both filed on Feb. 26, 1988 and now abandoned. Application Ser. No. 07/641,617 is a continuation of application Ser. No. 07/347,637, filed May 5, 1989, now abandoned. Application Ser. No. 07/737,899 is a continuation of application Ser. No. 07/363,138, filed Jun. 8, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/219,279, filed Jul. 15, 1988, now abandoned. Application Ser. No. 07/739,143 is a continuation-in-part of applications Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, and Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned. All of the above referenced priority applications are incorporated herein by reference in their entirety.

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Detail Description Paragraph - DETX (13):

[0062] Plant proteins do not require N-linked oligosaccharides for correct sorting into vacuoles (35,37,38). Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48). Sorting of Gal-A to the lysosome is likely to occur by the well-characterized mannose-6-phosphate receptor pathway in mammalian cells. We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi network. This signal appears to function in plant cells, presumably for vacuolar

localization.

Detail Description Paragraph - DETX (312):
[0315] 46 Melchers, L. S., Sela-Buurlage, M. B., Vloemans, S. A., Woloshuk, C. P., Van Roekel, J. S. C., Pen, J., Van den Elzen, P. J. M., Comelissen, B. J. C. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and .beta.-1,3-glucanase in transgenic plants. Plant Mol. Biol. 21:583, 1993.

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DOCUMENT-IDENTIFIER: US 20040064850 A1

TITLE: Antifungal polypeptide and methods for controlling plant pathogenic fungi

PUBLICATION-DATE: April 1, 2004

INVENTOR-INFORMATION:

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child 09103489 19980624 US

parent division-of 08627706 19960329 US GRANTED

parent-patent 5773696 US

US-CL-CURRENT: 800/279, 435/320.1 , 435/419 , 435/69.1 , 514/12 , 530/370 , 536/23.6

ABSTRACT:

An antifungal polypeptide, AlyAFP, that controls fungal damage to plants is provided. DNA encoding this polypeptide can be cloned into vectors for transformation of plant-colonizing microorganisms or plants, thereby providing a method of inhibiting fungal growth on plants. The polypeptide can be formulated into compositions that can be used to control undesired fingi on plants and elsewhere.

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Summary of Invention Paragraph - BSTX (17):

[0015] A number of publications have discussed the use of plant and bacterial glucanases, chitinases, and lysozymes to produce transgenic plants exhibiting increased resistance to various microorganisms such as fungi. These include EP 0 292 435, EP 0 290 123, WO 88/00976, U.S. Pat. No. 4,940,840, WO 90/07001, EP 0 392 225, EP 0 307 841, EP 0 332 104, EP 0 440 304, EP 0 418 695, EP 0 448 511, and WO 91/06312. The use of osmotin-like proteins is discussed in WO 91/18984.

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PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040023281 A1

TITLE: Production of lysosomal enzymes in plants by transient expression

PUBLICATION-DATE: February 5, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Turpen, Thomas H.	Vacaville	CA	US	
Kumagai, Monto H.	Kailua	HI	US	
Pogue, Gregory P.	Vacaville	CA	US	
Erwin, Robert L.	Davis	CA	US	
Grill, Laurence K.	Vacaville	CA	US	

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RELATED-US-APPL-DATA:

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parent continuation-in-part-of 09316572 19990521 US ABANDONED

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parent continuation-of 08324003 19941014 US GRANTED

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parent-patent 5811653 US

child 08176414 19931229 US

parent continuation-in-part-of 07997733 19921230 US ABANDONED

child 08324003

parent continuation-in-part-of 08184237 19940119 US GRANTED

parent-patent 5589367 US

child 08184237 19940119 US

parent continuation-in-part-of 07923692 19920731 US GRANTED

parent-patent 5316931 US

child 07923692 19920731 US

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07600244 19901022 US

parent continuation-of 07310881 19890217 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160766 19880226 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160771 19880226 US ABANDONED

US-CL-CURRENT: 435/6, 435/208 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

The invention relates to .alpha.-galactosidase truncated at the carboxy terminus and the production of enzymatically active recombinant human and animal lysosomal enzymes involving construction and expression of recombinant expression constructs comprising coding sequences of human or animal lysosomal enzymes in a plant expression system. The plant expression system provides for post-translational modification and processing to produce a recombinant gene product exhibiting enzymatic activity. The invention is demonstrated by working examples in which transgenic tobacco plants express recombinant expression constructs comprising human glucocerebrosidase nucleotide sequences. The invention is also demonstrated by working examples in which transfected tobacco plants express recombinant viral expression constructs comprising human .alpha. galactosidase nucleotide sequences. The recombinant lysosomal enzymes produced in accordance with the invention may be used for a variety of purposes, including but not limited to enzyme replacement therapy for the therapeutic treatment of human and animal lysosomal storage diseases.

RELATED APPLICATIONS

[0001] The present application is a continuation of U.S. patent application Ser. No. 09/993,059, filed Nov. 13, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/626,127, filed Jul. 26, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/316,572, filed May 21, 1999, which is a continuation of application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438, which is a continuation-in-part of application Ser. No. 08/176,414, filed on Dec. 29, 1993, now U.S. Pat. No. 5,811,653, which is a continuation-in-part of application Ser. No. 07/997,733, filed Dec. 30, 1992, now abandoned. application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438 is also a continuation-in-part of application Ser. No. 08/184,237, filed Jan. 19, 1994, now U.S. Pat. No. 5,589,367, which is a continuation-in-part of application Ser. No. 07/923,692, filed Jul. 31, 1992, now U.S. Pat. No. 5,316,931, which is a continuation-in-part of applications

Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, application Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned, and application Ser. No. 07/739,143, filed Aug. 1, 1991, now abandoned. application Ser. No. 07/600,244 is a continuation of application Ser. No. 07/310,881, filed Feb. 17, 1989, now abandoned, which is a continuation-in-part of applications Ser. No. 07/160,766 and Ser. No. 07/160,771, both filed on Feb. 26, 1988 and now abandoned. Application Ser. No. 07/641,617 is a continuation of application Ser. No. 07/347,637, filed May 5, 1989, now abandoned. application Ser. No. 07/737,899 is a continuation of application Ser. No. 07/363,138, filed Jun. 8, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/219,279, filed Jul. 15, 1988, now abandoned. Application Ser. No. 07/739,143 is a continuation-in-part of applications Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, and Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned.

---------- KWIC ---------

Detail Description Paragraph - DETX (46):

[0096] Plant proteins do not require N-linked oligosaccharides for correct sorting into vacuoles (35,37,38). Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48). Sorting of Gal-A to the lysosome is likely to occur by the well-characterized mannose-6-phosphate receptor pathway in mammalian cells. We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi network. This signal appears to function in plant cells, presumably for vacuolar localization.

Detail Description Paragraph - DETX (327):

[0339] 46. Melchers, L. S., Sela-Buurlage, M. B., Vloemans, S. A., Woloshuk, C. P., Van Roekel, J. S. C., Pen, J., Van den Elzen, P. J. M., Comelissen, B. J. C. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and .quadrature.-1,3-glucanase in transgenic plants. Plant Mol. Biol. 21:583, 1993.

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TITLE: Production of lysosomal enzymes in plants by transient expression

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INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Turpen, Thomas H.	Vacaville	CA	US	
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Erwin, Robert L.	Davis	CA	US	
Grill, Laurence K.	Vacaville	CA	US	

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DATE FILED: June 23, 2003

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child 09626127 20000726 US

parent continuation-in-part-of 09316572 19990521 US ABANDONED

child 09316572 19990521 US

parent continuation-of 08324003 19941014 US GRANTED

parent-patent 5977438 US

child 08324003 19941014 US

parent continuation-in-part-of 08176414 19931229 US GRANTED

parent-patent 5811653 US

child 08176414 19931229 US

parent continuation-in-part-of 07997733 19921230 US ABANDONED

child 08324003

parent continuation-in-part-of 08184237 19940119 US GRANTED

parent-patent 5589367 US

child 08184237 19940119 US

parent continuation-in-part-of 07923692 19920731 US GRANTED

parent-patent 5316931 US

child 07923692 19920731 US

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07641617 19910116 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07737899 19910726 US ABANDONED

child 07923692 19920731 US

parent continuation-in-part-of 07739143 19910801 US ABANDONED

child 07600244

parent continuation-of 07310881 19890217 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160766 19880226 US ABANDONED

child 07310881 19890217 US

parent continuation-in-part-of 07160771 19880226 US ABANDONED

child 07641617

parent continuation-of 07347637 19890505 US ABANDONED

child 07737899

parent continuation-of 07363138 19890608 US ABANDONED

child 07363138 19890608 US

parent continuation-in-part-of 07219279 19880715 US ABANDONED

child 07739143

parent continuation-in-part-of 07600244 19901022 US ABANDONED

child 07739143

parent continuation-in-part-of 07641617 19910116 US ABANDONED

child 07739143

parent continuation-in-part-of 07737899 19910726 US ABANDONED

US-CL-CURRENT: 800/280, 435/208 , 435/235.1 , 435/419 , 536/23.2 , 800/284

ABSTRACT:

The invention relates to .alpha.-galactosidase truncated at the carboxy terminus and the production of enzymatically active recombinant human and animal lysosomal enzymes involving construction and expression of recombinant expression constructs comprising coding sequences of human or animal lysosomal enzymes in a plant expression system. The plant expression system provides for post-translational modification and processing to produce a recombinant gene product exhibiting enzymatic activity. The invention is demonstrated by working examples in which transgenic tobacco plants express recombinant expression constructs comprising human glucocerebrosidase nucleotide sequences. The invention is also demonstrated by working examples in which transfected tobacco plants express recombinant viral expression constructs comprising human .alpha. galactosidase nucleotide sequences. The recombinant lysosomal enzymes produced in accordance with the invention may be used for a variety of purposes, including but not limited to enzyme replacement therapy for the therapeutic treatment of human and animal lysosomal storage diseases.

RELATED APPLICATIONS

[0001] The present application is a continuation of U.S. patent application Ser. No. 09/993,059, filed Nov. 13, 2001, which is a continuation-in-part of U.S. patent application Ser. No. 09/626,127, filed Jul. 26, 2000, which is a continuation-in-part of U.S. patent application Ser. No. 09/316,572, filed May 21, 1999, which is a continuation of application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438, which is a continuation-in-part of application Ser. No. 08/176,414, filed on Dec. 29, 1993, now U.S. Pat. No. 5,811,653, which is a continuation-in-part of application Ser. No. 07/997,733, filed Dec. 30, 1992, now abandoned. Application Ser. No. 08/324,003, filed Oct. 14, 1994, now U.S. Pat. No. 5,977,438 is also a continuation-in-part of application Ser. No. 08/184,237, filed Jan. 19, 1994, now U.S. Pat. No. 5,589,367, which is a continuation-in-part of application Ser. No. 07/923,692, filed Jul. 31, 1992, now U.S. Pat. No. 5,316,931, which is a continuation-in-part of applications Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, application Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned, and application Ser. No. 07/739,143, filed Aug. 1, 1991, now abandoned. Application Ser. No. 07/600,244 is a continuation of application Ser. No. 07/310,881, filed Feb. 17,1989, now abandoned, which is a continuation-in-part of applications Ser. No. 07/160,766 and Ser. No. 07/160,771, both filed on Feb. 26, 1988 and now abandoned. Application Ser. No. 07/641,617 is a continuation of application Ser. No. 07/347,637, filed May 5, 1989, now abandoned. Application Ser. No. 07/737,899 is a continuation of application Ser. No. 07/363,138, filed Jun. 8, 1989, now abandoned, which is a continuation-in-part of application Ser. No. 07/219,279, filed Jul. 15, 1988, now abandoned. Application Ser. No. 07/739,143 is a continuation-in-part of applications Ser. No. 07/600,244, filed Oct. 22, 1990, now abandoned, Ser. No. 07/641,617, filed Jan. 16, 1991, now abandoned, and Ser. No. 07/737,899, filed Jul. 26, 1991, now abandoned.

---------- KWIC ---------

Detail Description Paragraph - DETX (46):

[0107] Plant proteins do not require N-linked oligosaccharides for correct sorting into vacuoles (35,37,38). Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a

CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48). Sorting of Gal-A to the lysosome is likely to occur by the well-characterized mannose-6-phosphate receptor pathway in mammalian cells. We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi network. This signal appears to function in plant cells, presumably for vacuolar localization.

Detail Description Paragraph - DETX (333):

[0381] 46. Melchers, L. S., Sela-Buurlage, M. B., Vloemans, S. A., Woloshuk, C. P., Van Roekel, J. S. C., Pen, J., Van den Elzen, P. J. M., Cornelissen, B. J. C. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and .ident.-1,3-glucanase in transgenic plants. Plant Mol. Biol. 21:583, 1993.

PGPUB-DOCUMENT-NUMBER: 20030226175

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030226175 A1

TITLE: DNA regulatory elements associated with fruit development

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

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APPL-NO: 09/ 892635

DATE FILED: June 28, 2001

RELATED-US-APPL-DATA:

child 09892635 A1 20010628

parent continuation-in-part-of 09160351 19980925 US GRANTED

parent-patent 6284946 US

non-provisional-of-provisional 60060062 19970925 US

US-CL-CURRENT: 800/283, 435/193 , 435/320.1 , 435/419 , 536/23.2 , 800/287

ABSTRACT:

The present invention provides isolated and purified genes which are differentially expressed during banana fruit development, and the protein products of these genes. The present invention further provides DNA regulatory elements which are differentially expressed during banana fruit development, chimeric genes comprising these DNA regulatory elements operably linked to heterologous DNA molecules, and plants transformed with said chimeric genes, providing for controlled expression of said heterologous DNA molecules during the development and ripening of the fruit of said plants, or in response to exogenous ethylene signals in said plants. The present invention also provides a method for expression of a heterologous protein in fruit comprising transforming fruiting plants with one or more chimeric genes according to the present invention, exposing said fruit to an endogenous or exogenous ethylene signal, and harvesting fruit containing said heterologous protein. The method of the present invention may further comprise isolated the proteins produced by said method from the harvested fruit. In a particularly preferred embodiment, the heterologous protein is a therapeutic protein, which may be isolated from the harvested fruit, or consumed directly in the transformed fruit by a patient in need of said therapeutic protein.

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/160,351, filed Sep. 25, 1998, which is a continuation of provisional Application 60/060,062, filed on Sep. 25, 1997. This application claims priority of these aforementioned applications under 35 U.S.C. .sctn..sctn. 119 and 120 and the entire content of both of these priority applications is hereby incorporated by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX (90):

[0123] In addition to the N-terminal signal peptide, the banana P31 sequence is distinguished from other chitinase sequences by the presence of an 19 amino acid C-terminal extension (underlined in FIG. 8B). C-terminal propeptides (CTPPs) have been identified in a number of monocot and dicot polypeptides that direct proteins to the plant vacuole. Among others, CTPPs have been characterized in vacuolar lectins from barley and rice, and from vacuolar .beta.-1,3-glucanase and chitinase from tobacco (see, Bednarel, S. Y. (1992) Plant Molec. Biol. 20:133, for review). In general there is little sequence homology among plant vacuolar targeting sequences. However, weak homology can be detected between the C-terminal extension of P31 (SNILSMP) and vacuolar targeting sequences that have been characterized in the sweet potato storage protein sporamin (NPIRLP) (Linthorst, H. J. M. (1991) Crit. Rev. Plant Sci 10: 123) and in a 2S albumin from Brazil nut (NLSPMRCP) (Saalbach, G. et al. (1996) Plant Physiol. 112:975).

PGPUB-DOCUMENT-NUMBER: 20030177530

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030177530 A1

TITLE: (1 -> 3, 1 -> 4)-beta-glucanase of enhanced stability

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Varghese, Joseph Noozhumurry	Melbourne		AU	
Garrett, Thomas Peter John	Melbourne		AU	
Fincher, Geoffrey Bruce	Melbourne		AU	
Hoj, Peter Bordier	Melbourne		AU	
Chen, Lin	Melbourne		AU	

APPL-NO: 09/ 785246

DATE FILED: February 20, 2001

RELATED-US-APPL-DATA:

child 09785246 A1 20010220

parent continuation-of 08584008 19960111 US GRANTED

parent-patent 6277615 US

child 08584008 19960111 US

parent continuation-in-part-of PCT/AU94/00377 19940706 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PL 9821	1993AU-PL 9821	July 7, 1993

US-CL-CURRENT: 800/284, 435/200 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2

ABSTRACT:

A modified cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase is produced by the method of single point substitution in a native cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme, whereby the substitution:
a) maintains enzyme specificity by conserving the active site groove of the native cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme; and
b) effects increased thermostability over the native cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme by:
i) replacing glycine by proline or alanine in helices of the cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme, in order to stiffen the enzyme amino acid chain and reduce entropy of the unfolded enzyme;
ii) attaching negatively charged residues to N-termini of helices in the native cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme;
iii) introducing ion pairs into the native cereal (1.fwdarw.3,1.fwdarw.4)--.beta.-glucanase enzyme, to increase binding energy in the folded enzyme;
iv) replacing lysine by arginine in the cereal (1.fwdarw.3,1.fwdarw.4)-.be-

ta.-glucanase enzyme, and thereby preventing lysine glycation and increasing hydrogen bonding with other parts of the enzyme;
v) replacing, by glycine, an amino acid in the native cereal (1.fwdarw.3,1.fwdarw.4)-.beta.-glucanase enzyme in which the main chain torsion angle about the N and C.sup..alpha. atoms is greater than 0.degree.; or
vi) creating cysteine pairs in the native cereal (1.fwdarw.3,1.fwdarw.4)-.-beta.-glucanase enzyme which can form disulphide bonds across the C and N terminals.

---------- KWIC ---------

Detail Description Paragraph - DETX (42):

[0058] Two more steps are required for the mutant enzymes to be incorporated into barley and expressed in a spatially and temporally appropriate manner. These are construction of a barley glucanase gene with the appropriate control of expression, and the insertion of the gene into a viable barley plant. The sequence the EII gene, including the promoter regions and the coding region and the signal peptide has been determined (Wolf, 1991). Thus for correct expression of the mutant glucanases we will replace a portion of this gene by the corresponding portion of a mutant cDNA using the above methods. It is expected that transformation of barley, that is to regenerate a fertile transgenic barley plant, will be possible in the near future. Foreign or manipulated DNA can be integrated into the barley genome in a stable form (Lazzeri et al, 1991) and fertile plants can be regenerated from single protoplasts (Jahne et al, 1991a, b). Among the cereals related to barley, rice can now be routinely transformed, and transformation of both wheat and maize has been reported. Methods for effecting transformation of monocotyledonous plants such as barley using biolistic techniques are widely used, and whole plants of transgenic barley have been grown. Barley has recently been transformed using the biolistic microprojectile gun procedure (Wan and Lemaux, 1994).

PGPUB-DOCUMENT-NUMBER: 20030131376

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030131376 A1

TITLE: Nucleic acid sequences encoding cell wall-degrading enzymes and use to engineer resistance to fusarium and other pathogens

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Okubara, Patricia A.	Pullman	WA	US	
Blechl, Ann E.	Richmond	CA	US	
Hohn, Thomas M.	Chapel Hill	NC	US	
Berka, Randy M.	Davis	CA	US	

APPL-NO: 10/ 316754

DATE FILED: December 10, 2002

RELATED-US-APPL-DATA:

child 10316754 A1 20021210

parent division-of 09649747 20000828 US GRANTED

parent-patent 6521435 US

non-provisional-of-provisional 60224946 20000811 US

non-provisional-of-provisional 60151582 19990830 US

US-CL-CURRENT: 800/278, 435/196 , 435/320.1 , 435/419 , 435/69.1 , 536/23.2 , 800/320.3

ABSTRACT:

The invention is directed to nucleic acid sequences derived from Fusarium fungal genes which encode the cell wall-degrading enzymes glucanase, endochitinase, and exochitinase; isolated polypeptides having glucanase, endochitinase or exochitinase activity; recombinant nucleic acid molecules, vectors, and host cells comprising the nucleic acid sequences as well as methods for producing and using the polypeptides, including expression in plant cells to confer or enhance a plant's resistance to Fusarium and other pathogens.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Applications Nos. 60/224,946, filed Aug. 11, 2000 and 60/151,582, filed Aug. 30, 1999. The disclosure of each of said provisional application is incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (18):

[0018] Some of the chitinases and .beta.-1,3-glucanases produced by naturally occurring bacteria and fungi have anti-Fusarium properties (Mitchell and Alexander 1961; Michael and Nelson 1972, Chrif and Benhamou 1990). Glucanases and chitinases from plants can degrade isolated cell walls of Fusarium solani (Mauch et al. 1988). Chitinases from tobacco were inhibitory to the growth of F. oxysporum (Yun et al. 1996) and F. solani (Sela-Buurlage et al. 1993) in culture. Krishnaveni et al. (1999b) have described three chitinases from sorghum seeds that inhibit the growth of F. moniliforme. The synergistic action of chitinases and glucanases against fungal pathogens is widely reported (reviewed in Graham and Sticklen 1994, Van Loon 1997). For instance, Mauch et al. (1988) observed that a chitinase and a .beta.-1,3-glucanase from pea wee active against a wide range of fungi. Melchers et al (1994) reported the combined action of a Class V endochitinase plus a Class I .beta.-1,3-glucanase, both from tobacco, synergistically inhibited the growth of F. solani. Expression of a tobacco acidic chitinase with a tobacco .beta.-1,3-glucanase conferred resistance to F. oxysporum in tomato, whereas each protein had much less effect when expressed singly (Jongedijk et al. 1995). Likewise, the fungal pathogen Cercospora nicotiana was curtailed on tobacco expressing both a rice basic chitinase and an alfalfa acidic .beta.-1,3-glucanase (Zhu et al. 1994). The synergistic action of a barley Class II chitinase and a barley Class II b-1,3-glucanase conferred protection to tobacco against Rhizoctonia solani (Jach et al. 1995). This chitinase in combination with a barley ribosome inactivating protein also inhibited R. solani infection.

Summary of Invention Paragraph - BSTX (24):

[0024] A majority of antifungal genes that have been examined both in vitro and in planta are of plant origin. To our knowledge, there are two examples of genes from fungi that exhibit antifungal activity. Endochitinases from the parasitic fungus Trichoderma harzianum conferred activity against Alternaria alternata and B. cinerea in transgenic tobacco, and against A. solani and Rhizoctonia solani in transgenic potato (Lorito et al. 1998). Terakawa et al. (1997) observed protection of transgenic tobacco against Sclerotinia sclerotiorum and B. cinerea, using a chitinase gene from the fungus Rhizopus oligosporus. A chitinase from the bacteria Serratia marcescens showed antifungal activity when expressed in tobacco (Suslow et al. 1988).

Detail Description Paragraph - DETX (240):

[0302] The present invention is particularly useful in wheat and other cereals. A number of methods of transforming cereals have been described in the literature. For instance, reliable methods for stable transformation of wheat, including the highly-regenerable cultivars such as the hard white spring wheat Bobwhite, are described (Vasil, et al., 1992, 1993; Weeks, et al., 1993; Becker et al., 1994; Nehra et al. 1994, Blechl and Anderson, 1996). U.S. Pat. Nos. 5,650,558 and 5,914,450 to Blechl et al. describe transformation of wheat and non-wheat cereal plants, which patents are incorporated herein by reference. Chen et al. (1998) introduced a rice chitinase gene under control of the 35S promoter into wheat. It was expressed in the first generation, but was subsequently silenced in progeny plants. Jensen et al. (1998) introduced a modified heat-stable 1,3-1,4-glucanase gene under control of its own promoter into barley and obtained plants that expressed the gene in germinating aleurone and scutellum. Bliffeld et al. (1998) introduced a barley seed class II chitinase gene under control of the maize Ubiquitin-1 promoter into wheat. Two lines of transgenic plants containing this construct showed increased resistance to infection by the powdery mildew-causing fungus Erysiphe graminis. Transgenic maize regenerants have been described by Fromm, et al., Bio/Technology 8:833-839 (1990) and Gordon-Kamm, et al., Plant Cell 2:603-618

(1990)). Similarly, oats (Sommers, et al., Bio/Technology 10:1589-1594 (1992)), sorghum (Casas, et al., Proc. Nat'l Acad. Sci. USA 90:11212-11216 (1993)), rice (Li, et al., Plant Cell Rep. 12:250-255 (1993)), barley (Yuechun & Lemaux, Plant Physiol. 104:37-48 (1994)), and rye (Castillo, et al., Bio/Technology 12:1366-1371 (1994)) have been transformed via bombardment. Transformation of rice is described by Toriyama, et al., Bio/Technology 6:1072-1074 (1988), Zhang, et al., Theor. Appl. Gen. 76:835-840 (1988), and Shimamoto, et al., Nature 338:274-276 (1989).

Detail Description Paragraph - DETX (408):
[0424] Jensen, L. G., O. Politz, O. Olsen, K. K. Thomsen and D. Von Wettstein (1998). Inheritance of a codon-optimized transgene expressing heat stable (1,3-1,4)--glucanase in scutellum and aleurone of germinating barley. Hereditas 129: 215-225

Detail Description Paragraph - DETX (409):
[0425] Jondedijk, E., H. Tigelaar, J. S. C. van Roekel, S. A. Bres-Vloemans, I. Dekker, P. J. M. van den Elzen, B. J. C. Cornelissen, and L. S. Melchers (1995). Synergistic activity of chitinases and .beta.-1,3-glucanases enhances fungal resistance in transgenic tomato plants. Euphytica 85: 173-180

Detail Description Paragraph - DETX (410):
[0426] Jutidamrongphan, W., J. B. Anderson, C. Mackinnon, J. M. Manners, R. S. Simpson and K. J. Scott (1991). Induction of .beta.-1,3-glucanase in barley in response to infection by fungal pathogens. Mol. Plant-Microbe Inter. 4: 234-238

Detail Description Paragraph - DETX (422):
[0438] Malehorn, D. E., K. J. Scott and D. M. Shah (1993). Structure and expression of a barley acidic .beta.-glucanase gene. Plant Mol. Biol. 22: 347-360

Detail Description Paragraph - DETX (470):
[0486] Zhu, Q., E. A. Maher, S. Masoud, R. A. Dixon and C. J. Lamb (1994). Enhanced protection against fungal attack by constitutive co-expression of chitinase and glucanase genes in transgenic tobacco. Bio/Tech. 12: 807-812

PGPUB-DOCUMENT-NUMBER: 20030129180

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030129180 A1

TITLE: Products and methods for treating microbial infections

PUBLICATION-DATE: July 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Baker, Keith Homer	West Chester Township	OH	US	
Grayling, Rowan Andrew	Loveland	OH	US	

APPL-NO: 10/ 209203

DATE FILED: July 31, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60309329 20010801 US

US-CL-CURRENT: 424/94.61, 435/200

ABSTRACT:

A method for treating athlete's foot involves treating both the infected skin and the shoes of a subject suffering from athlete's foot. Skin treatment compositions and shoe treatment compositions can be used concurrently to treat the infection.

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 60/309,329, filed Aug. 1, 2001.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (30):

[0027] Enzymes that degrade these structural components of cell walls are known, with specific types hydrolyzing the polymers either within the polymer chain (endo-acting) or from either end of the polymer (exo-acting). Preferred enzymes are those that hydrolyze the polymers in an endo fashion, since these more rapidly break up the long polymer chains that maintain the structural integrity of the cell wall. Furthermore, such enzymes may act synergistically, that is, their effect in combination is more than the sum of their effects individually. Synergism is thought to be especially pronounced with chitinases and glucanases, since the respective polymers are important in most fungal cell walls. This invention takes advantage of rapid hydrolysis of fungal cell wall polymers by endo-acting enzymes, especially by chitinases and glucanases in combination, to degrade dermatophyte cell walls, and thereby inhibit or prevent dermatophyte growth, or both.

PGPUB-DOCUMENT-NUMBER: 20030106095

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030106095 A1

TITLE: Production of lysosomal enzymes in plants by transient expression

PUBLICATION-DATE: June 5, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Garger, Stephen J.	Vacaville	CA	US	
Turpen, Thomas H.	Vacaville	CA	US	
Kumagai, Monto H.	Davis	CA	US	

APPL-NO: 10/ 103327

DATE FILED: March 20, 2002

RELATED-US-APPL-DATA:

child 10103327 A1 20020320

parent continuation-of 09993059 20011113 US PENDING

child 09993059 20011113 US

parent continuation-in-part-of 09626127 20000726 US PENDING

US-CL-CURRENT: 800/280, 435/207 , 435/410 , 435/468 , 536/23.2

ABSTRACT:

The invention relates to .alpha.-galactosidase truncated at the carboxy terminus and the production of enzymatically active recombinant human and animal lysosomal enzymes involving construction and expression of recombinant expression constructs comprising coding sequences of human or animal lysosomal enzymes in a plant expression system. The plant expression system provides for post-translational modification and processing to produce a recombinant gene product exhibiting enzymatic activity. The invention is demonstrated by working examples in which transgenic tobacco plants express recombinant expression constructs comprising human glucocerebrosidase nucleotide sequences. The invention is also demonstrated by working examples in which transfected tobacco plants express recombinant viral expression constructs comprising human .alpha. galactosidase nucleotide sequences. The recombinant lysosomal enzymes produced in accordance with the invention may be used for a variety of purposes, including but not limited to enzyme replacement therapy for the therapeutic treatment of human and animal lysosomal storage diseases.

RELATED APPLICATIONS

[0001] This present application is a continuation of U.S. application Ser. No. 09/993,059, filed Nov. 13, 2001, which is a continuation-in-part of U.S. application Ser. No. 09/626, 127, filed Jul. 26, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX (46):

[0106] Plant proteins do not require N-linked oligosaccharides for correct sorting into vacuoles (35,37,38). Some vacuolar proteins (osmotin, thaumatin, chitinase-I, glucanase-I and a barley lectin), contain sorting information in a CTPP of 7 to 22 AA in length. For several of these proteins secreted isoforms are synthesized without a CTPP domain. In other cases, experimental deletion of the CTPP results in secretion of the recombinant protein to the IF (45-48). Sorting of Gal-A to the lysosome is likely to occur by the well-characterized mannose-6-phosphate receptor pathway in mammalian cells. We hypothesize that a redundant sorting signal may exist in this carboxy-domain that also serves to reduce enzymatic activity in the ER lumen, golgi and trans-golgi network. This signal appears to function in plant cells, presumably for vacuolar localization.

Detail Description Paragraph - DETX (301):

[0347] 46. Melchers, L. S., Sela-Buurlage, M. B., Vloemans, S. A., Woloshuk, C. P., Van Roekel, J. S. C., Pen, J., Van den Elzen, P. J. M., Cornelissen, B. J. C. Extracellular targeting of the vacuolar tobacco proteins AP24, chitinase and .quadrature.-1,3-glucanase in transgenic plants. Plant Mol. Biol. 21:583, 1993.

PGPUB-DOCUMENT-NUMBER: 20030097682

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030097682 A1

TITLE: Genetically modified plants with enhanced resistance to fungal diseases and a method of production thereof

PUBLICATION-DATE: May 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chye, Mee Len	Hong Kong		HK	
Zhao, Kai-Jun	Beijing		CN	

APPL-NO: 10/ 300819

DATE FILED: November 20, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60331749 20011120 US

US-CL-CURRENT: 800/279, 435/320.1 , 435/419 , 435/6

ABSTRACT:

The present invention discloses genetically modified plants, such as potato plants. The plants are more resistant to a pathogen of interest following transformation of plant cells with a chimeric gene comprising a chitinase gene and .beta.-1,3-glucanase gene. The invention also provides a method of enhancing the resistance of plants to pathogens by introducing a Brassica chitinase gene encoding two or more chitin-binding domains and .beta.-1,3-glucanase gene and expressing the chitinase gene and .beta.-1,3-glucanase gene.

[0001] This application is entitled to and claims priority benefit to U.S. provisional application Serial No. 60/331,749, filed Nov. 20, 2001, which is incorporated herein by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (9):
[0008] Also, chitinases act synergistically with .beta.-1,3-glucanases in inhibiting fungal growth (Mauch et al., 1988, Plant Physiol. 88:936-942; Zhu et al., 1994, Bio/Technology 12:807-812; Jach et al., 1995, Plant J. 8:97-109)

Summary of Invention Paragraph - BSTX (18):
[0016] In a specific embodiment, the present invention further provides transgenic plant comprising a chitinase and a .beta.-1,3-glucanase. In a specific embodiment, the chitinase and the .beta.-1,3-glucanase are encoded in separate vectors. In another specific embodiment, the chitinase and the .beta.-1,3-glucanase are encoded in the same vector. In a specific embodiment, the chitinase has two or more chitin-binding domains. In another specific

embodiment, the chitinase is BjCHI1 and the .beta.-1,3-glucanase is HbGLU.

Brief Description of Drawings Paragraph - DRTX (14):

[0035] FIG. 12 shows glucanase and chitinase assays on transgenic potato lines: A. Glucanase assays measured in optical density at 500 nm using crude protein from pBI121-transformant and transgenic R.sub.0 lines transformed with pBj47; B. Chitinase assays measured in optical density at 550 nm using crude protein pBI121-transformant and transgenic Ro lines transformed with pBj47. Error bars represent standard deviations of three separate experiments.

Detail Description Paragraph - DETX (44):

[0079] 5.4 Transgenic Plants Expressing Chitinase and .beta.-1,3-Glucanase

Detail Description Paragraph - DETX (45):

[0080] A transgenic plant with the ability to express a plant chitinase or .beta.-1,3-glucanase polypeptide may be engineered by transforming a plant cell with a gene construct comprising a sequence encoding a plant chitinase and .beta.-1,3-glucanase protein or polypeptide. In one embodiment, a plant promoter is operably associated with a sequence encoding the desired plant chitinase or .beta.-1,3-glucanase protein or polypeptide. As used herein, the term "Operably associated" or "operably linked" refers to an association in which the regulatory regions (e.g., promoter, enhancer) and the nucleic acid sequence to be expressed are covalently joined and positioned in such a way as to permit transcription, and under the appropriate condition, translation. In a preferred embodiment of the present invention, the associated promoter is a strong and non tissue- or developmental-specific plant promoter (e.g., a promoter that strongly expresses in many or all plant tissue types). Examples of such strong, "constitutive" promoters include, but are not limited to, the CaMV 35S promoter (Odell et al., 1985, Nature 313:810-812), the T-DNA mannopine synthetase promoter, and their various derivatives. In another preferred embodiment, an inducible or repressible promoter is used to express the chitinase and/or .beta.-1,3-glucanase of interest in a plant, for example, a tet operator promoter as described in Weinmann et al., 1994, The Plant Journal 5:559-569; or a glucocorticoid-inducible promoter as described in McNellis et al., 1998, The Plant Journal 14:247-257; or an ethanol inducible promoter as described in Caddick et al., 1998, Nature Biotechnology 16:177-180. See, also, Gatz, 1995, Methods In Cell Biology 50:411-424, which describes inducible and repressible gene expression systems for plants.

Detail Description Paragraph - DETX (46):

[0081] In one embodiment of the invention, a chitinase and/or .beta.-1,3-glucanase is expressed in a plant so that the chitinase and/or .beta.-1,3-glucanase polypeptide will be localized in the apoplastic space. The chitinase and/or .beta.-1,3-glucanase may be directed to the apoplastic space, when expressed in a plant, by expressing the chitinase and/or .beta.-1,3-glucanase polypeptide as a fusion protein together with a peptide that acts as a signal or transporter so that chitinase and/or .beta.-1,3-glucanase is localized in the apoplastic space of the transgenic plant. A variety of signal or transporter peptides can be used, for example, the PR1b signal sequence as described in Lund et al., 1992, Plant Molecular Biology 18:47-53; or the PR-1a, b and c signal sequences as described in Pfitzner et al., 1987, Nucleic Acids Research 15:4449-4465. A fusion protein comprising a signal or transporter peptide and a chitinase and/or .beta.-1,3-glucanase polypeptide may be constructed by linking polynucleotides specific for each component to each other (e.g., the polynucleotides are linked in frame) so that the desired fusion protein is made when the fusion polynucleotide is expressed in a transgenic plant. A skilled artisan would know how to construct a polynucleotide useful for expressing a chitinase and/or

.beta.-1,3-glucanase in the apoplastic space of a transgenic plant.

Detail Description Paragraph - DETX (59):
[0094] 5.7 Transgenic Plants Expressing an Engineered Chitinase and .beta.-1,3-Glucanase Polynucleotides

Detail Description Paragraph - DETX (60):
[0095] Transgenic plants are generated that express an engineered chitinase and .beta.-1,3-glucanase gene. A transgenic plant expressing a chitinase and .beta.-1,3-glucanase is less susceptible to the pathogenic effects of the pathogen of interest. Transgenic plants may be made using any of the techniques known in the art as described for plant chitinase and .beta.-1,3-glucanase expressing transgenic plants, supra.

Detail Description Paragraph - DETX (61):
[0096] Transgenic plants expressing one or more chitinase and .beta.-1,3-glucanase gene polynucleotides capable of rendering said plants more resistant to a pathogen of interest may be from any plant species, plant genus, plant family, plant order, plant class, plant division of the kingdom of plants. See, e.g., U.S. Pat. Nos. 5,889,189; 5,869,720; 5,850,015; 5,824,842; PP10,742; PP10,704; PP10,682, which recite plant species, genuses, families, orders, classes and divisions in which the chitinase and .beta.-1,3-glucanase genes may be used.

Detail Description Paragraph - DETX (67):
[0102] A polynucleotide construct capable of directing the expression of an engineered chitinase and .beta.-1,3-glucanase gene product in a transgenic plant of interest is constructed using general recombinant DNA and cloning techniques known in the art of biotechnology, see, e.g., Sambrook et al., supra; Ausubel et al., supra. Such a polynucleotide construct typically comprises a polynucleotide sequence that encodes an engineered chitinase and .beta.-1,3-glucanase gene product and one or more regulatory polynucleotide sequence. Regulatory sequences useful for the polynucleotide construct of the invention include, but are not limited to, a promoter, an enhancer, an intron, a splice donor, a splice acceptor, a polyadenylation sequence, a RNA stability regulating sequence, or an element of any one of the above (e.g., promoter elements including, but not limited to, a TATA box).

Detail Description Paragraph - DETX (70):
[0105] In order to facilitate the proper processing of the engineered chitinase and .beta.-1,3-glucanase gene product, it may be necessary to include a nucleotide stretch that encodes a peptide sequence necessary for such processing. For example, a peptide sequence which is recognized by and functional in the transgenic host plant, for example, to facilitate the entry of the chitinase and .beta.-1,3-glucanase gene product into the endoplasmic reticulum may be necessary, i.e., signal sequence.

Detail Description Paragraph - DETX (73):
[0108] Another way of testing a trangenic plant made using the methods of the invention is by testing for necrosis inducing activity, for example, as described in Mahe et al., 1998, J. Peptide Res. 52:482-494. Thus, one can express an engineered chitinase and .beta.-1,3-glucanase gene in a transgenic plant and infect the transgenic plant with the pathogen of interest. For example, when applying a pathogen to the transgenic plant expressing the engineered chitinase and .beta.-1,3-glucanase gene, one would observe clear necrosis or severe spreading necrosis in the wild-type plant but not in a transgenic plant of the plant line from which the transgenic plant was derived.

Detail Description Paragraph - DETX (95):

[0129] 6.2 Generation of Transgenic Potato Plants Carrying The Hevea .beta.-1,3-Glucanase and Brassica Chitinase BjCHI1 Constructs

Detail Description Paragraph - DETX (124):

[0158] Transgenic potato lines were tested for chitinase and .beta.-1,3-glucanase activity assays and were compared to pBI121 transformed potato (FIG. 12). Glucanase assays (FIG. 12A) using crude protein from pBI121-transformant and transgenic potato lines pBj47-P.sub.7, pBj47-P8 and pBj47-P.sub.10 show that the transgenic lines had higher levels of .beta.-1,3-glucanase activity than that of the pBI121 transformant. Chitinase assays (FIG. 12B) using crude protein show that transgenic potato lines pBj47-P.sub.7, pBj47-P.sub.8 and pBj47-P.sub.10 show higher levels of chitinase activities than of the pBI121 transformant. Activities detected in the pBI121 transformant are due to the presence of endogenous potato .beta.-1,3-glucanases and chitinases.

US-PAT-NO: 6693227

DOCUMENT-IDENTIFIER: US 6693227 B1

TITLE: Inducibile plant promoters

DATE-ISSUED: February 17, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Gittins; John Robert	Havant	N/A	N/A	GB
James; David John	Maidstone	N/A	N/A	GB
Hiles; Elizabeth Rachel	Aylesford	N/A	N/A	GB

APPL-NO: 09/ 415161

DATE FILED: October 5, 1999

PARENT-CASE:

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is a continuation of PCT/GB98/01000 filed Apr. 3, 1998, which in turn claims priority to U.K. Patent Application No. 9707193.0, filed Apr. 9, 1997, and to UK Patent Application No. 9711233.8, filed May 31, 1997, all of which are incorporated by reference in their entirety.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9707193	April 9, 1997
GB	9711233	May 31, 1997

US-CL-CURRENT: 800/287, 435/320.1 , 435/419 , 435/468 , 435/6 , 536/24.1 , 800/279 , 800/283 , 800/286 , 800/302 , 800/315

ABSTRACT:

The invention relates to a recombinant polynucleotide comprising a promoter sequence being an inducible promoter obtainable from apple. The promoter sequence is preferably activated in response to which agents are specific to ripening fruit and is most preferably the apple .beta.-galactosidase (ABG1) promoter. Vectors form a further part of the invention. Also provided are host plant cells, plus methods of producing transgenic plants and fruit which incorporate antisense RNA capable of down-regulating genes involved in ripening or peptides or proteins improving fungal, insect, bacterial, viral, herbicidal, nematode, or arachnid resistance. Such transgenic plants and fruit have storage and pest-resistance properties superior to non-transgenic varieties.

9 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

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Detailed Description Text - DETX (26):

In genetically improved transgenic apple plants, the storage qualities of the fruit may be improved by the expression of transgenes driven by the ripening-specific promoters. Using antisense or co-suppression strategies to down-regulate apple genes involved in ripening (e.g. genes involved in ethylene biosynthesis or cell wall degradation), the ripening process may be delayed, thus improving the storage life of the fruit. This strategy has successfully been applied to tomatoes to produce a marketable product. To combat post-harvest losses of fruit due to fungal rots, fruit-specific expression of fungal-resistance transgenes (e.g. .beta.-1,3-glucanases, chitinases) may be more effective than treatment with chemical fungicides because the anti-fungal molecules will be located in every cell rather than applied as a thin coating to the fruit skin. Therefore, even slightly damaged fruit will be less susceptible to rots. Such transformants will have advantages over existing systems. For instance certain traditional apple varieties have poor storage qualities (e.g. Queen Cox) which is a major commercial drawback. Genetic manipulation using the promoters described above provides a means to control the ripening process through targeted down-regulation of the genes involved. This concept, which is impossible using existing strategies, has previously been proved only in tomato and melon. Delayed fruit ripening caused by the expression of transgenes under the control of the is fruit-specific ABG1 and AAS promoters is likely to increase the storage life of fruit and boost profits for the industry.

US-PAT-NO: 6660915

DOCUMENT-IDENTIFIER: US 6660915 B2

TITLE: Low lipoxygenase 1 barley

DATE-ISSUED: December 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Douma; Anna Christina	Zeist	N/A	N/A	NL
Doderer; Albert	Zoetermeer	N/A	N/A	NL
Cameron-Mills; Verena	Valby	N/A	N/A	DK
Skadhauge; Birgitte	Birkeroed	N/A	N/A	DK
Bech; Lene Moelskov	Smoerum	N/A	N/A	DK
Schmitt; Natalie	Den Haag	N/A	N/A	NL
Heistek; Jolanda Carolina	Vlaardingen	N/A	N/A	NL
van Mechelen; Johannes Reinier	Amsterdam	N/A	N/A	NL

APPL-NO: 09/ 751687

DATE FILED: December 29, 2000

US-CL-CURRENT: 800/320, 426/11 , 426/64 , 426/7 , 435/183 , 435/185 , 800/278 , 800/298

ABSTRACT:

Barley plants having reduced lipoxygenase-1 enzyme activity are provided, for example, barley plants expressing mutant LOX-1 protein. The barley plants of the invention are useful in the production of plant products such as malt and brewed beverages, particularly beer, having increased stability and reduced T2N potential.

11 Claims, 29 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 29

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Other Reference Publication - OREF (10):
Jensen, L. et al., "Inheritance of a Codon-Optimized Transgene Expressing Heat Stable (1,3-1,4)-.beta.-glucanase in Scutellum and Aleurone of Germinating Barley", Hereditas, vol. 129, pp. 215-225 (1998).

Other Reference Publication - OREF (39):
Gilpin, M. et al., "The Effect of Reduced PSI-K Protein Levels on Photosynthesis in Transgenic Barley", Photosynthesis: Mechanisms and Effects, vol. IV, pp. 2983-2986 (1998).

US-PAT-NO: 6653280

DOCUMENT-IDENTIFIER: US 6653280 B2

TITLE: Antifungal polypeptide AlyAFP from Alyssum and methods for controlling plant pathogenic fungi

DATE-ISSUED: November 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liang; Jihong	Chesterfield	MO	N/A	N/A
Shah; Dilip Maganlal	Chesterfield	MO	N/A	N/A
Wu; Yonnie Shun	Chesterfield	MO	N/A	N/A
Rosenberger; Cindy Annette	Ballwin	MO	N/A	N/A

APPL-NO: 09/ 829381

DATE FILED: April 9, 2001

PARENT-CASE:

This is a divisional of application Ser. No. 09/103,489, filed Jun. 24, 1998, now U.S. Pat. No. 6,215,048, which is a divisional of Ser. No. 08/627,706, filed Mar. 29, 1996, now U.S. Pat. No. 5,773,696.

US-CL-CURRENT: 514/2, 424/404 , 514/12 , 530/324 , 530/370

ABSTRACT:

An antifungal polypeptide, AlyAFP, that controls fungal damage to plants is provided. DNA encoding this polypeptide can be cloned into vectors for transformation of plant-colonizing microorganisms or plants, thereby providing a method of inhibiting fungal growth on plants. The polypeptide can be formulated into compositions that can be used to control undesired fungi on plants and elsewhere.

20 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 12

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Brief Summary Text - BSTX (17):

A number of publications have discussed the use of plant and bacterial glucanases, chitinases, and lysozymes to produce transgenic plants exhibiting increased resistance to various microorganisms such as fungi. These include EP 0 292 435, EP 0 290 123, WO 88/00976, U.S. Pat. No. 4,940,840, WO 90/07001, EP 0 392 225, EP 0 307 841, EP 0 332 104, EP 0 440 304, EP 0 418 695, EP 0 448 511, and WO 91/06312. The use of osmotin-like proteins is discussed in WO 91/18984.

US-PAT-NO: 6627736

DOCUMENT-IDENTIFIER: US 6627736 B1

TITLE: Pap mutants that exhibit anti-viral and/or anti-fungal activity in plants

DATE-ISSUED: September 30, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tumer; Nilgun E.	Belle Mead	NJ	N/A	N/A

APPL-NO: 09/ 639456

DATE FILED: August 15, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 09/005,273, filed Jan. 9, 1998, now U.S. Pat. No. 6,137,030, which is a continuation of PCT/US96/11546, filed Jul. 11, 1996, which is a continuation-in-part of U.S. application Ser. No. 08/500,611, filed Jul. 11, 1995, now U.S. Pat. No. 5,756,322, and application Ser. No. 08/500,694, filed Jul. 11, 1995, now U.S. Pat. No. 5,880,329.

US-CL-CURRENT: 530/370, 435/254.2 , 435/440 , 435/468

ABSTRACT:

Disclosed are PAP mutants having reduced phytotoxicity compared to wild-type PAP, and which confer broad spectrum resistance to viruses and/or fungi in plants. One group of PAP mutants is characterized by at least one amino acid substitution in the N-terminus of mature PAP, such as the Glycine 75 residue or the Glutamic acid 97 residue; two groups of additional PAP mutants are characterized by truncations in the N-terminal region of mature PAP and truncations or amino acid substitutions in the C-terminal region of mature PAP, respectively; and a further group are enzymatically inactive which still exhibit anti-fungal properties. Also disclosed are DNA molecules encoding the PAP mutants, mutant PAP DNA constructs, and transgenic seed and plants containing the DNAs. Further disclosed are methods for identifying PAP mutants having reduced phytotoxicity, as well as isolated and purified PAP mutants identified by the method.

51 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (8):

In addition to the studies on virus resistance in plants, RIPs have been studied in conjunction with fungal resistance. For example, Logeman et al., Bio/Technology 10:305-308 (1992), report that a RIP isolated from barley

endosperm provided protection against fungal infection to transgenic tobacco plants. The combination of barley endosperm RIP and barley class-II chitinase has provided synergistic enhancement of resistance to Rhizoctonia solani in tobacco, both in vitro and in vivo. See, e.g., Lea et al., supra; Mauch et al., supra; Zhu et al., supra; and Jach et al., The Plant Journal 8:97-109 (1995). PAP, however, has not shown antifungal activity in vitro. See Chen et al., Plant Pathol. 40:612-620 (1991), which reports that PAP has no effect on the growth of the fungi Phytophthora infestans, Colletotrichum coccodes, fusarium solani, fusarium sulphureum, Phoma foreata and Rhizoctonia solani in vitro.

US-PAT-NO: 6610494

DOCUMENT-IDENTIFIER: US 6610494 B2

TITLE: Solid-phase activity assay for biologically active substance

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Marquardt; Ronald R.	Winnipeg, Manitoba	R3T	N/A	CA
Hao; Xiao	2W2	N/A	V5Z 1A1	CA
Wang; Guojie	Vancouver, British	N/A	210095	CN
N/A	Columbia		210095	CN
Zhang; Zhiqun	Nanjing			CA
Gan; Zhibo	Nanjing			
	Winnipeg, Manitoba R3& 3M2			

APPL-NO: 09/ 180819

DATE FILED: May 20, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of priority of PCT Application No. PCT/US97/07983, filed May 13, 1997, which claims benefit of priority under 119(e) U.S. Provisional Patent Application No. 60/017,659, filed May 14, 1996, both of which are incorporated herein in their entirety.

PCT-DATA:
APPL-NO: PCT/US97/07983
DATE-FILED: May 13, 1997
PUB-NO: WO97/43438
PUB-DATE: Nov 20, 1997
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/7.1, 435/28 , 435/5 , 435/6 , 435/7.9 , 436/172 , 436/518 , 514/12

ABSTRACT:

A method of detecting via a solid-phase assay the amount of biological activity, identity and/or the quantity of a biologically active substance is disclosed. The method utilizes the biological activity of the substance itself to provide the method of detection. The method provides competitive and noncompetitive assays.

6 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC ---------

Detailed Description Text - DETX (88):

REFERENCES Bailey and Nevalainen, 1981. Induction, Isolation and Testing of Stable Trichoderma reesei Mutants with Improved Production of Solubilizing Cellulase. Enzyme Microbiol. Technol. 3:153-157. Bolger and Checovich, 1994. A new protease activity assay using fluorescence polarization. BioTechniques 17:585-589. Bourne and Pierce, 1970. .beta.-Glucan and .beta.-Glucanase in Brewing. J.Inst.Brew. 76:328-335. Buhler, 1991. Double-antibody Sandiwich Enzyme-linked Immunosorbent Assay for Quantisation of Endoglucanase I of Trichoderma reesei. Appl. and Environ. Microbi. 57:3317-3321. Cullmann, 1990. Interaction of .beta.-lactamase inhibitors with various .beta.-lactamases. Chemotherapy 36:200. Denalt, et al., 1978. A Simple Reducing Sugar Assay for Measuring .beta.-Glucanase in Malt of Various Microbial Enzyme Preparations. J. Amer.Soc.Brew.Chem. 36:18-23. Edney, 1986. Application of a Simple Radial Gel Diffusion Assay for Endo-.beta.-glucanase Activity in Dietary Enzyme Supplements. Poultry Sci. 65:72-77. Green, 1963. Biochem. J. 89:585-591. Headon, 1993. Activity Analysis of Enzymes Under Field Conditions. In Enzymes in Animal Nutrition; Wenk, C., Boessinger, M., Eds., Inst. Nutztierwisserschaften Zurich, Switzerland, 233-240. Henrissart, et al., 1985. Synergism of Cellulases from Trichoderma reesei in the Degradation of Cellulase. Biotechnol. 3:722-726. IUBMB. (1992) Enzyme Nomenclature 1992: Recommendations of the Nomenclature Committees of IUBMB on the Nomenclature and Classification of Enzymes. Academic Press, New York. Kemeny and Challacombe, 1980. ELISA and Other Solid Phase Immunoassays, Theoretical and Practical Aspects. J. Wiley and Sons Ltd. New York, N.Y. 1988 Lorand, 1981. Proteolytic Enzymes Part C. Method in Enzymology 80. Academic Press, N.Y. Martin and Bamforth, 1983. Application of Radial Diffusion Assay for the Measurement of .beta.-Glucanase Activity in Malt. J.Inst.Brew. 89:34-37. McCleary and Shameer, 1987. Assay of Malt .beta.-Glucanase Using Azo-barley Glucan: An Improved Precipitant. J.Inst.Brew. 93:87-90. McClear and Glennie-Holmes, 1985. Enzymatic Quantification of (1-3)(1-4) .beta.-D-Glucan in Barley and Malt. J.Inst.Brew 91:285-295. Miller, et al., 1989. Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 A resolution. Science, 246:1149. Nieves, et al., 1995. Quantisation of Acidothermus cellulolyticus E1 Endoglucanase and Thermomonospora fusca E3 Exoglucanase Using Enzyme-linked Immunosorbent Assay (ELISA). Appl. Biochem. Biotechnol. 51/52:211-223. Ondetti and Cushman, 1982. Enzymes of the renin-angiotensin system and their inhibitors. Ann. Rev. biochem. 51:283-308. Pasztai, 1989. Lectins in Toxicants of Plant Origin, Volume III Protein and Amino Acids, (Editor) Peter R. Cheeke, CRC Press, Boca Raton Fla., 33431. pgs 29-71) Rossomando, 1990. Measurement of enzyme activity. Method in Enzymology 182:38-50. Academic Press, N.Y. Sevier, 1976. Anal. Biochem. 74:592-596. Stoll, and Blanchard, 1990. Buffers: principle and practice. Method in Enzymology 182:24-38, Academic Press, N.Y. Twining, 1984. Fluorescein isothiocyanate-labeled casein assay for proteolytic enzymes. Anal. Biochem. 143:30-34. Voss, et al., 1996. Detection of protease activity using a fluorescence-enhancement globular substrate. BioTechnigues 20:286-291. Wirth and Wolf, 1992. Micro-plate Colourimetric Assay for Endo-acting, Cellulase, Xylanase, Chitinase,1,3-.beta.-Glucanase and Amylase Extracted from Forest Soil Horizons. Soil Biol.Biochem. 24:511-519. Wong, 1991. Chemistry of Protein Conjugational and Cross-linking, CRC Press, Inc. Boca-Raton, Fla. Wood and Bhat, 1988. Methods for Measuring Cellulase Activities. Methods Enzymol. 160:87-112. Yu, 1995. Adsorption and Desorption of Cellulase Components During the Hydrolysis of a Steam-exploded Birch Substrate. Biotech.Appl.Biochem. 21:203-216. Zollner, 1993. Handbook of Enzyme

Inhibitors (2.sup.nd edition) (VCH Publishers, New York).

US-PAT-NO: 6573361

DOCUMENT-IDENTIFIER: US 6573361 B1

TITLE: Antifungal proteins and methods for their use

DATE-ISSUED: June 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bunkers; Greg J.	Wildwood	MO	N/A	N/A
Liang; Jihong	Chesterfield	MO	N/A	N/A
Mittanck; Cindy A.	Ballwin	MO	N/A	N/A
Seale; Jeffrey W.	Ballwin	MO	N/A	N/A
Wu; Yonnie S.	Vacaville	CA	N/A	N/A

APPL-NO: 09/ 732210

DATE FILED: December 7, 2000

PARENT-CASE:

This application claims priority of the U.S. provisional application, serial No. 60/169,340, filed on Dec. 7, 1999, and of the U.S. provisional application, serial No. 60/169,513, filed on Dec. 7, 1999.

US-CL-CURRENT: 530/324, 435/252.3 , 435/254.1 , 435/254.7 , 435/320.1 , 435/410 , 435/69.1 , 435/69.7 , 530/300 , 536/23.6

ABSTRACT:

A novel protein was isolated from Fusarium culmorum and characterized. The protein, termed FCWP1, demonstrated significant antifungal activity against several fungal species. Mutations in proteolytic consensus sequences contained within FCWP1 improved the stability of its antifungal activity. In addition, a class of proteins related to FCWP1 was identified and characterized. This class is made up of ribosomal proteins and displayed similar values for pI and molecular weight. A representative number of proteins from this class were tested and found to have significant antifungal activities.

The antifungal proteins disclosed herein are useful in controlling fungal infections in plants. Transgenic plants may be produced that are more resistant to fungal infections relative to non-transgenic plants of the same species. Alternatively, the proteins may be applied to plants exogenously.

6 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Brief Summary Text - BSTX (10):

A number of publications have described methods of using antifungal proteins from plants and bacteria in transgenic plants. The antifungal proteins used in

these methods include glucanases, chitinases, osmotin-like proteins, and lysozymes produced in transgenic plants exhibiting increased resistance to various microorganisms (EP 0 292 435, EP 0 290 123, WO 88/00976, U.S. Pat. No. 4,940,840, WO 90/07001, EP 0 392 225, EP 0 307 841, EP 0 332 104, EP 0 440 304, EP 0 418 695, EP 0 448 511, WO 91/06312, WO 93/05153, and WO 25 91/18984).